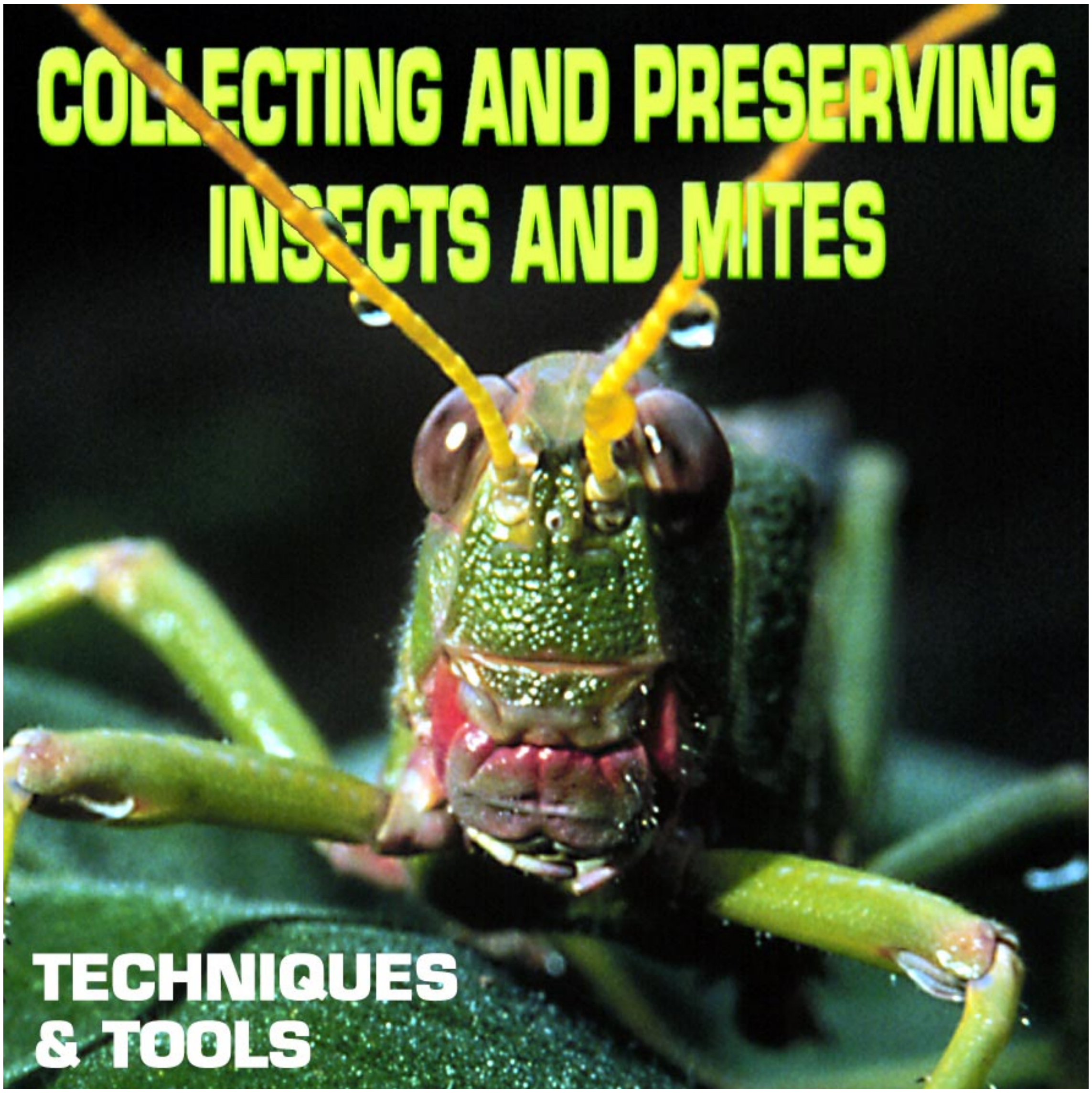
COLLECTING AND PRESERVING INSECTS AND MITES



TECHNIQUES & TOOLS

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Contents

Introduction

The Class Arthropoda, which includes insects, spiders, mites, and their relatives, is without question the most successful group of organisms on the planet. Insects alone account for nearly 55% of all species known to science (Barrowclough 1992). Spiders, mites and insects inhabit every terrestrial habitat on the planet and play a major role in the evolution and maintenance of biotic communities. They are the primary pollinators of flowering plants; they are important consumers and recyclers of decaying organic matter; and they are integral components in the foodwebs of vertebrates and other invertebrates. For these reasons, and many others, the study of insects and their relatives is of increasing importance as society faces increased challenges to preserve and enhance environmental quality, reduce pesticide usage, increase crop productivity, control food costs, and increase trade in the global community. Pest species are responsible for enormous economic losses annually, attacking crops and ornamental plants, causing damage to our food and clothing, and vectoring diseases that effect cultivated plants, our pets and livestock, and ourselves. The damage cause by pests species is far outweighed by the positive effects of beneficial species. Pollinators ensure the production of fruit, parasitoids and predators help control pest species, some species contain chemicals of pharmaceutical value, and a large number of species contribute to the decomposition and recycling of dead and decaying matter.

Because of the damage inflicted by pest species, increased knowledge of these organisms has the potential to save lives and money. Correct identification of a newly detected pest or disease vector is of utmost importance because the scientific name of an organism is the key to all known information about its morphology, its behavior and life history, and its potential threat to human welfare.

The behavior of insects and mites can be observed most easily in their natural environments. However, many species, especially the smaller ones, must be collected and properly preserved before they can be identified. Because correct identification seldom is easy, it is important that specimens be preserved in the best condition possible. The identification of a particular insect or mite usually requires examination of minute details of its anatomy with the aid of a hand lens or microscope. Some specimens may require dissection or even study with the electron microscope. If these details on a specimen are concealed, missing, or destroyed because of improper handling or preservation, identification is made difficult or impossible, and information about the species to which it belongs cannot be made available. Therefore, adequate preservation and proper labeling of specimens are essential to their identification.

The methods used to collect insects and mites are dictated by the ultimate goal of the samples collected. Insects may be collected as a hobby for personal enjoyment of their diversity and beauty. They may be collected in conjunction with school courses on biology or entomology. Specific insects groups may be sampled to assess or measure biodiversity

* This manual is an updated and modified version of the USDA Misc. Publication no. 1443 published by the Agricultural Research service in 1986 and Edited by George C. Steyskal, William L. Murphy, and Edna M. Hoover.

to help identify appropriate areas to be included in reserves. Aquatic species may be used to detect changes in water quality. Pest species may be sampled to assess presence/absence or abundance in order to determine whether control measures are necessary. Specific groups or species may be collected to acquire material for biological, physiological, ecological, molecular, and systematic studies.

This manual provides a summary of the methods and techniques used by professionals and amateurs alike to collect and preserve specimens for study. While many of the methods covered here, such as pinning, have changed very little in the last hundred years, other techniques have become available only in the last few years or decades with advancing technologies. Older manuals such as Steyskal et al. (1986), Martin (1977) and Upton (1991) while still useful will not cover such these as preservation for molecular studies. In addition, most of these older publications are now out of print and may be difficult to find.

What to Collect

Because of their incredible diversity, insects, mites, and other related groups vary widely as to their proper collecting requirements and methods. In the following sections, we will explore some of the many recommended techniques and look at the varied equipment used by collectors. The emphasis will be on insects and mites, but much of what is included here will also pertain to other related groups such as spiders.

Which species and how many specimens to collect depends on the purpose for which the material is intended. For hobbyists and students, small samples are usually adequate. However, when important pest insects and mites need to be identified, they should be collected in series if at all feasible. A sample of 20 specimens should be considered the minimum, and even larger numbers may be desirable. If adults and immatures are present, specimens should be collected of all life stages. Excess specimens can be discarded or exchanged, but it is not always possible to collect additional specimens when needed. Frequently insects and mites cannot be identified accurately from immature stages, and it is then necessary to rear them to the adult stage to obtain a precise identification. Photographers should collect the specimens they photograph if positive identification is desired; minute, critical diagnostic characters often are not depicted in photographs. If specimens are destined for display cases that portray them in their natural habitats, it may be important to collect a sample of the host plant for the display.

Many persons starting a collection attempt to collect every specimen they find. Biology students in high school and college are often required to collect specimens from as many orders or groups as possible. The experience and knowledge gained in making a general collection are of value in helping the collector decide on a specialty. However, with so many different kinds of insects from which to choose—over 100,000 described species in North America alone—most persons find that as their skills and interests increase, concentrating eventually on 1 or 2 of the major insect or mite groups is desirable. Specimens other than those in a chosen group may still be collected for exchange with other collectors.

References: Lewis & Taylor 1965; Seber 1973; Barrowclough 1992.

Part 1. Equipment and Collecting Methods

1.1 Basic Equipment

Collecting methods may be divided into two broad categories. In the first the collector actively searches out



Fig. 1. A field collecting kit.

the insects, using nets, aspirators, beating sheets, or whatever apparatus suits his or her particular needs. In the second, the collector participates passively and permits traps to do the work. Both approaches may be used simultaneously, and both are discussed in the following pages. Using a variety of collecting methods will help to maximize the number of specimens taken, especially when briefly visiting an interesting area.

While picking up insects by hand is simple and sometimes effective, their size, mobility, and the possibility of being bitten or stung usually dictates that various kinds of equipment and special methods are needed. Those described here have general application; it is expected that the collector will make some adaptations to fit his or her own purposes and resources. In fact, as experience collecing increases, or the target group becomes more focussed, the use of specialized techniques increases. For additional information, especially concerning the use of specialized techniques, consult the list of references.

References (general): Arnett, 1985; Balogh 1958; Banks 1909; Banks et al. 1981; Bland & Jacques 1978; Borror et al.; British Museum 1974; Cantrall 1939-40; Cantrall 1941; Chu 1949; Edmunds & McCafferty 1978; Foote 1948; Klots 1932; Knudsen 1966; Knudsen 1972; Kogan & Herzog 1980; Lehker & Deay 1969; Lincoln & Sheals 1979; McNutt 1976; Martin 1977; Nicholls 1970; Norris 1966; Oldroyd 1958; Peterson 1964; Service 1976; Southwood 1979; Stein 1976; Upton, 1991; USDA 1970; USDA 1966-70; Urquhart 1965; Wagstaffe & Fidler 1955.

The equipment used to assemble a general insect or mite collection need not be elaborate or expensive. In many instances, a collecting net (see below) and several killing bottles (see p. 5) will suffice; however, additional items will permit more effective sampling of a particular fauna. Many collectors carry a bag (fig. 1) or wear a vest in which they store equipment. The following items usually are included in the general collector’s bag:

(1) Forceps. Fine, lightweight forceps are recommended; if sharp-pointed forceps are used, care must be taken not to puncture specimens. If possible, grasp specimens with the part of the forceps slightly behind the points.

(2) Vials containing alcohol or other preservatives (see p. 21).

(3) Killing bottles of various sizes.

(4) Small boxes or containers for storing specimens after their removal from killing bottles. These may be made of cardboard, plastic, or metal and should be partly filled with soft tissue or cloth to keep specimens from rolling about. Do not use cotton because specimens become entangled in the fibers and may become virtually impossible to extricate without damage.

(5) Small envelopes for temporary storage of delicate specimens and/or gelcaps for tiny specimens.

(6) One or more aspirators (see p. 7-8).

(7) Absorbent tissue for use in killing bottles and aspirators.

(8) Notebook and writing equipment for jotting down notes and label data.

(9) A strong knife for opening galls, seed pods, twigs, etc and a pair of scissors for cutting labels.

(10) A small, fine brush (camel’s hair is best) for picking up minute specimens. Moisten the tip; tiny specimens will adhere to it and may be transferred to a killing bottle or vial.

(11) Bags for storing plant material, rearing material, or Berlese samples. For collecting much plant material, a botanist’s vasculum or tin box is advisable.

(12) A hand lens.

This list may be modified according to the special kinds of insects or mites to be collected. A small digging tool or trowel may be useful for collecting insects from soil or for gathering Berlese samples and a heavy knife or small hatchet for searching under bark or in decaying logs. A plant press should be available to prepare plant specimens for determination or as voucher specimens, especially when leaf-mining insects are being studied. When collecting at night, have a flashlight or headlamp; the latter is especially useful because it leaves the hands free.

Much of the equipment listed above may be obtained from around the home or from ordinary sources like a drug store, but equipment especially designed for insect collecting often must be bought from special supply houses. If there is a local company, their address may usually be found in the yellow pages of telephone directories under “Biological Laboratory Supplies” or “Laboratory Equipment and Supplies.” The faculty members of a local university’s biology or entomology department or curators at a nearby museum are usually willing to help and in the best position to recommend a supplier in the area. Professional journals also sometimes carry advertisements for equipment suppliers.

1.2 Collecting Nets

Collecting nets come in three basic forms: Aerial, sweeping, and aquatic. The first is designed especially for

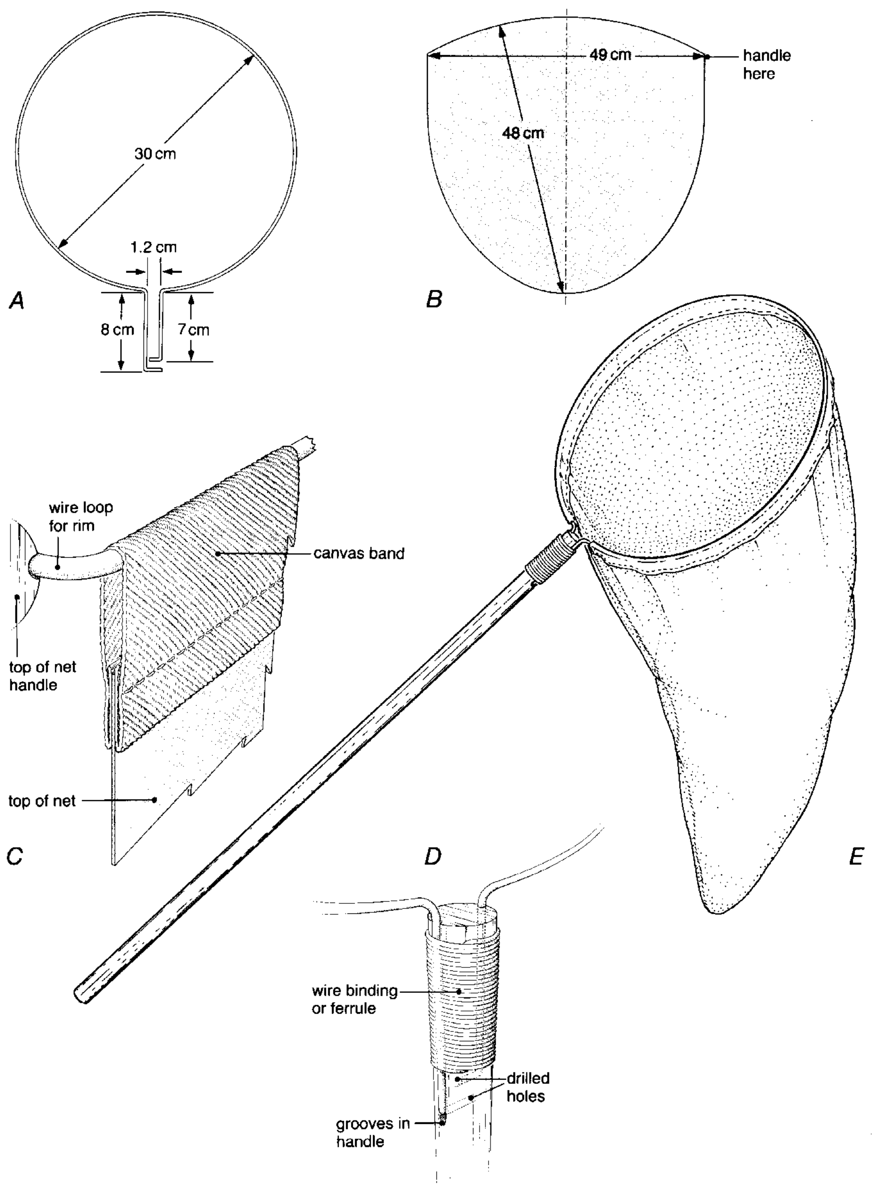


Fig. 2. Collecting Net

collecting butterflies and other flying insects. Both the bag and handle are relatively lightweight. The sweeping net is similar to the aerial net but is stronger and has a more durable bag to withstand being dragged through dense vegetation. Aquatic nets are used for gathering insects from water and are usually made of metal screening or heavy scrim with a canvas band affixed to a metal rim. A metal handle is advisable because wooden ones may deteriorate after repeated wetting. The net you choose depends on the kind of insects or mites you wish to collect.

Several kinds of nets, including collapsible models with interchangeable bags, are available from biological supply houses, but anyone with a little mechanical ability can make a useful net. The advantage of a homemade net is that the size and shape can be adapted to the needs of the user, to the kind of collecting intended, and to the material available, which need not be expensive. These materials include—

(1) Piece of heavy (8-gage) steel wire for the rim, bent to form a ring 30-38 cm in diameter (fig. 2, A). Small nets 15 cm or so in diameter sometimes are useful, but nets larger than 38 cm are too cumbersome for most collecting.

(2) Dacron or other strong, light fabric through which air can flow freely. Brussels netting is best but may be difficult to obtain; otherwise nylon netting, marquisette, organdy, or good quality cheesecloth can be used, but the last snags easily and is not durable. The material should be folded double and should be 1.5-1.75 times the rim diameter in length (fig. 2, B). The edges should be double-stitched (French seams).

(3) Strip of muslin, light canvas, or other tightly woven cloth long enough to encircle the rim. The open top of the net bag is sewn between the folded edges of this band to form a tube through which the wire rim is inserted (fig. 2, C).

(4) Straight hardwood dowel about 19 mm in diameter and 105-140 cm long (to suit the collector). For attachment of the rim to the handle, a pair of holes of the same diameter as the wire are drilled opposite each other to receive the bent tips of the wire, and a pair of



Fig. 3. A truck equipped with a large net.

grooves as deep and as wide as the wire are cut from each hole to the end of the dowel to receive the straight part of the wire (fig. 2, D).

(5) Tape or wire to lash the ends of the rims tightly into the grooves in the end of the handle. This may be electrician's plastic tape or fiber strapping tape commonly used for packaging. If wire is used, the ends should be bound with tape to secure them and to keep them from snagging. A close-fitting metal sleeve (ferrule) may be slipped over the rim ends and held in place with a small roundheaded screw instead of tape or wire lashing.

After the net has been placed on the rim, the ends of the band should be sewn together and the rim ends fastened to the handle. The other end of the handle should be filed to remove sharp edges. The net is then ready for use (fig. 2, E).

Efficient use of a net is gained only with experience. Collection of specimens in flight calls for the basic stroke—swing the net rapidly to capture the specimen, then follow through to force the insect into the very bottom of the bag. Twist the wrist as you follow through so the bottom of the bag hangs over the rim; this will entrap the specimen. If the insect alights on the ground or other surface, it may be easier to use a downward stroke, quickly swinging down on top of the insect. With the rim of the net in contact with the ground to prevent the specimen from escaping, hold the tip of the bag up with one hand. Most insects will fly or crawl upward into the tip of the bag, which can then be flipped over the rim to entrap the specimen.

Sweeping the net through vegetation, along the sand and seaweed on beaches, or up and down tree trunks will catch many kinds of insects and mites. The aerial net may be used in this way, but the more durable sweeping net is recommended for such rough usage. After sweeping with the net, a strong swing will bring anything in the bag to the bottom, and then by immediately grasping the middle of the net with the free hand, the catch will be confined to a small part of the bag. Only the most rugged sweeping net may be used through thistles or brambles. Even some kinds of grasses, such as sawgrass, can quickly ruin a net. Burs and sticky seeds are also a serious problem.

The catch may be transferred from the bag to a killing jar in one of several ways. Single specimens are transferred most easily by lightly holding them in a fold of the net with one hand while inserting the open killing jar into the net with the other. While the jar is still in the net, cover the opening until the specimen is stupefied; otherwise, it may escape before the jar can be removed from the net and closed. To prevent a butterfly from damaging its wings by fluttering in the net, squeeze the thorax gently through the netting when the butterfly's wings are closed. Experience will teach you how much pressure to exert; obviously, pinching small specimens of any kind is not recommended. When numerous specimens are in the net after prolonged sweeping, it may be desirable to put the entire tip of the bag into a large killing jar for a few minutes to stun the insects. They may then be removed and desired specimens placed separately into a killing jar, or the entire mass may be dumped into a killing jar for later sorting. These methods of mass collecting are especially adapted to obtaining small insects not readily recognizable until the catch is sorted under a microscope.

Removal of stinging insects from a net may be a problem. They will often crawl toward the rim of the bag and may be made to enter a killing jar held at the point where they crawl over the rim. However, many insects will fly as soon as they reach the rim, and a desired specimen may be lost. A useful method is to trap the insect in a fold of the net, carefully keeping a sufficient amount of netting between fingers and insect to avoid being stung. This fold of the net can then be inserted into the killing jar to stun the insect. After a few moments, it should be safe to remove the insect from the net and transfer it to a killing jar. If the stunned insect clings to the net and does not fall readily into the jar, use forceps or pry the insect loose with the jar lid or a small stick—not with your fingers.

Aerial nets made of dacron or nylon may be used to sweep insects from water if an aquatic net is not at hand. The netting will dry quickly if swept strongly through the air a few times; however, it should not be used again until thoroughly dry, or other specimens, especially butterflies, may be ruined. A number of special modifications are necessary to adapt a net for aquatic collecting.

For specialized collecting, nets can be attached to the ends of beams that are rotated about their midlength by a motor drive. Nets also can be adapted to be towed by or mounted on vehicles (fig. 3) (Peck and Cook, 1992).

References: Dresner 1970; Johnson 1950; Rogers & Smith 1977; Rudd & Jensen 1977; Takeda et al. 1962; Williams & Miine 1935; vehicle-mounted net: Almand et al. 1974; Barnard 1979; Grigarick 1959; Harwood 1961; Hill 1971; Holzapfel et al. 1978; Kronblad & Lundberg 1978; Landin 1976; McNutt 1976; Noyes, 1982; Rudd & Jensen 1977; Torre-Bueno 1937; Traver 1940.

1.3 Killing Jars or Bottles

Effective collecting of insects and related groups usually requires that the specimens be killed so that they may be properly mounted and studied. The most widely

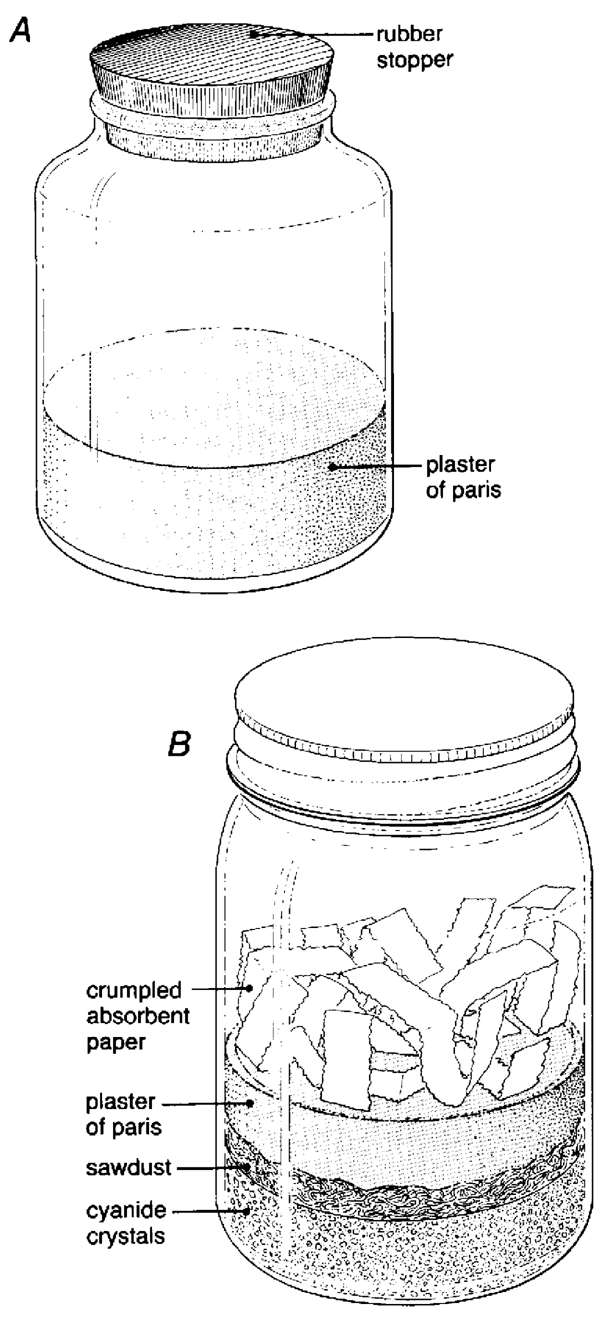


Fig. 4. Killing jars

employed method for killing collected specimens is the killing jar (bottle). Any heavy, wide-mouthed glass jar or bottle with a tight-fitting stopper or metal screw top may be used. Tops that may be removed with only a quarter turn often are preferred but may not be obtained readily. The killing agent used may be any of various liquids or solids. Liquid killing agents generally are considered to be slower acting but safer to use than solids such as cyanide, but some of them are known to accumulate in human tissue after repeated or prolonged exposure. Despite its extreme toxicity, cyanide is a noncumulative poison, and brief exposure to the fumes, as inevitably occurs when opening jars to insert or remove specimens, is not believed to reault in any permanent harm. Never deliberately inhale the fumes, even momentarily. All killing agents are to some extent hazardous to human health. All killing jars or bottles should be clearly labeled "**POISON**" and should be kept away from children or persons who may be unaware of their potential danger.

When not in use, killing jars should be stored in a safe place away from children and pets where they are not liable to accidental breakage. Cyanide jars should not be stored in an area such as a bedroom where any accidental leakage could expose someone to fumes. Remember that killing agents can be as effective against humans as they are against insects and that care and caution in their construction and use are essential.

1.4 Liquid Killing Agents

Jars for use with liquid killing agents are prepared in one of two ways. One way (fig. 4, A) is to pour about 2.5 cm of plaster of paris mixed with water into the bottom of the jar and allow the plaster to dry. Enough of the killing agent is then added to saturate the plaster; any excess should be poured off. This kind of jar can be recharged merely by adding more killing agent. The second method is to place a wad of cotton or other absorbent material in the bottom of a jar, pour enough liquid killing agent into the jar to nearly saturate the absorbent material, and then press a piece of stiff paper on it or a cardboard cut to fit the inside of the jar tightly. The paper or cardboard acts as a barrier between the insect and the killing agent, keeping the latter from evaporating too rapidly and also preventing the specimen from becoming entangled in loose fibers.

Among the liquid killing agents are ethyl acetate ($CH_3CO_2 \cdot C_2H_5$), ether (diethyl ether, $C_2H_5 \cdot O \cdot C_2H_5$), chloroform ($CHCI_3$), and ammonia water ($NH_4OH$ solution). Ethyl acetate is most widely used. All of these chemicals are extremely volatile and flammable and should never be used near fire. Children should only use them under adult supervision.

Ethyl acetate is regarded by many as the most satisfactory liquid killing agent. Its fumes are less toxic to humans than those of the other substances. Although it usually stuns insects quickly, it kills them slowly. Specimens that appear dead may revive if removed from the killing jar too soon, but a compensating advantage is that most specimens may be left in an ethyl acetate killing jar for several days and still be limp. If the ethyl acetate is allowed to evaporate from the specimens, they will harden. Killing jars with ethyl acetate are preferred by many entomologists, especially for infrequent use.

Ether and chloroform are both extremely volatile and

flammable and should not be used near an open flame or lighted cigarette. Their high volatility makes them serviceable in a killing jar for only a short time. Perhaps the greatest hazard with chloroform is that even when stored in a dark-colored jar, it eventually forms the extremely toxic gas phosgene (carbonyl chloride, COCI2). Chloroform, however, is useful when other substances cannot be obtained. It stuns and kills quickly but has the disadvantage of stiffening specimens.

Ethyl Alcohol (ethanol or ETOH) is widely used to kill small Coleoptera adults, small Hymenoptera, and many immature insects and soft-bodied insects. It is most commonly used at 70-80% concentration and many workers add 5% glacial acetic acid ("acetic alcohol") which helps penetration of the alcohol into the specimen and leaves specimens more relaxed. Isopropyl alcohol (rubbing alcohol) may also be used, and may be easier to find and purchase than Ethanol. However, Ethanol is preferred for most applications. Ethanol is used commonly in Berlese funnels and similar traps.

Liquid ammonia is irritating to humans, and in general is not a particularly effective killing agent for most insects. However, it is highly recommended for use in small vials for dispatching microlepidoptera, and it has been used with variable success in blacklight traps, again for Lepidoptera. Specimens killed in ammonia tend to stay in a relaxed condition much longer than those killed by cyanide, allowing greater ease of spreading. Ammonia is readily available from many sources. Ammonium carbonate, a solid but volatile substance, also can be used.

1.5 Solid Killing Agents

The solid killing agents most often used in killing jars are the cyanides—potassium cyanide (KCN), sodium cyanide (NaCN), or calcium cyanide [Ca(CN)2]. Handle all cyanides with extreme care. They are dangerous, rapid-acting poisons with no known antidote. If even a single grain touches the skin, wash immediately with water. To avoid handling the cyanide and having to find a safe place to store or dispose of surplus crystals, you may be able to find a chemist, pharmacist, or professional entomologist to make the killing jar for you. If this is not feasible, use utmost care in following the instructions given here.

To make a cyanide killing jar or bottle, place a layer (about 15 mm) of cyanide crystals in the bottom (fig. 4, B). Potassium cyanide is best; sodium cyanide is as effective but is hygroscopic, that is, it absorbs water and makes the jar wet; and calcium cyanide is seldom available. Cover the crystals with about 10 mm of sawdust and then add about 7 mm of plaster of paris mixed with water to form a thick paste, working quickly before the plaster solidifies. Then add crumpled absorbent paper to prevent water condensation on the inside glass surface. Instead of the plaster of paris, a plug of paper or cardboard may be pressed on top of the sawdust. Be sure that it fits tightly. When ready to use after a few hours, place several drops of water on the plaster or paper plug. In an hour or so, enough fumes of hydrocyanic acid will have been produced to make the jar operative. **Do not test this by sniffing the open jar**.

Every killing jar or bottle should be clearly and prominently labeled “POISON”. The bottom must be covered with tape, preferably cloth, plastic, or clinical adhesive tape, to cushion the glass against breakage and to keep its dangerous contents from being scattered if the container breaks.

Killing jars or bottles will last longer and give better results if the following simple rules are observed:

(1) Place a few narrow strips of absorbent paper in each jar or bottle to keep it dry and to prevent specimens from mutilating or soiling each other. Replace the strips when they become moist or dirty. This method is useful for most insects except Lepidoptera, which are too difficult to disentangle without damage.

(2) Do not leave killing jars in direct sunlight as they will sweat and rapidly lose their killing power.

(3) If moisture condenses in a jar, wipe it dry with absorbent tissue.

(4) Keep delicate specimens in separate jars so that larger specimens will not damage them.

(5) Do not allow a large number of specimens to accumulate in a jar unless it is to be used specifically for temporary storage.

(6) Do not leave insects in cyanide jars for more than a few hours. The fumes will change the colors of some insects, especially yellows to red, and specimens will generally become brittle and difficult to handle.

(7) If it is necessary to keep insects in killing jars for more than several hours, place the specimens in another container and store them in a refrigerator.

(8) Keep butterflies and moths in jars by themselves so that their hairs and scales will not ruin other kinds of insects.

(9) Never test a killing jar by smelling its contents.

(10) Old jars that no longer kill quickly should be recharged or disposed of by burning or burying. A cyanide

jar that has become dry may be reactivated by adding a few drops of water.

Spray-dispensed insecticides may be used, if not to kill specimens, to at least 'knock them down' into a container from which they may be picked up. If they are directed into a container topped with a funnel, they may be allowed to revive and treated further as desired (see Clark & Blom 1979).

References: Banks et al. 1981; Clark & Blom 1979; Frost 1958; Lindroth 1957; Pennington 1967; Preiss et al.; White 1964.

1.6 Aspirators and Suction Devices

The aspirator (fig. 5, A), known in England as a 'pooter,' is a convenient and effective device for collecting small insects and mites. The following materials are needed to construct an aspirator:

(1) Vial 2.5-5 cm in diameter and about 12 cm long.

(2) Two pieces of glass or copper tubing about 7 mm in diameter, one piece about 8 cm long and the other about 13 cm long.

(3) Rubber stopper with two holes in which the tubing will fit snugly.

(4) Piece of flexible rubber or plastic tubing about 1 meter long, with diameter just large enough to fit snugly over one end of shorter piece of stiff tubing.

(5) Small piece of cloth mesh, such as cheesecloth, and rubberband.

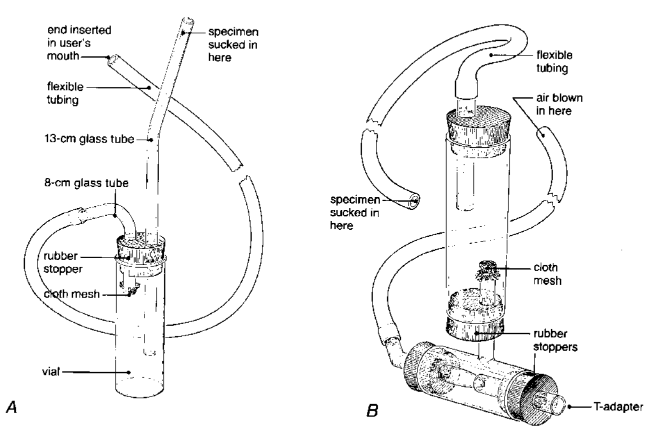


Fig. 5. Aspirators

To make an aspirator, bend the glass or copper tubes as in figure 5, A. In bending or cutting glass tubes, always protect your fingers by holding the glass between several layers of cloth. Obtain the advice of a chemist or laboratory technician for cutting and bending glass. Moisten one end of the longer tube and insert it through one of the holes in the rubber stopper. Moisten one end of the shorter tube, insert it through the other hole in the stopper, and using a rubberband fasten the cloth mesh over the end that was inserted through the stopper; this will prevent specimens from being sucked into the collector's mouth when the aspirator is used. Attach one end of the flexible tubing to the free end of this tube. The length, size, and amount of bend in the tubing will vary according to the user's needs. To complete the assembly, insert the rubber stopper into the vial. To use the aspirator, place the free end of the flexible tubing in the mouth, move the end of the longer glass tube close to a small specimen, and suck sharply. The specimen will be pulled into the vial.

Instead of using a vial, some workers prefer a tube (fig. 5, B). In either method, it is well to keep small pieces of absorbent tissue in the vial or tube at all times to prevent moisture from accumulating. Be cautioned that there is some danger of inhaling harmful substances or organisms when using a suction-type aspirator (see Hurd 1954).

Either the vial- or tubing-type aspirator (fig. 5, B) may be converted into a blow-type aspirator by removing the 13-cm glass tube (see fig. 5, A) and substituting a T—shaped attachment (fig. 5, B). The flexible tubing is attached to one arm of the 'T,' the opposite arm is left open, and the stem of the 'T' is inserted into the vial and covered with mesh. Upon blowing through the flexible tubing, a current of air passes across the 'T' and creates a partial vacuum in the vial, which produces the suction needed to draw specimens into the vial. This kind of aspirator eliminates the danger of inhaling small particles, fungus spores, or noxious fumes.

Aspirators with a squeeze bulb may sometimes be purchased, or if a valved bulb can be obtained, they may be constructed for use with either pressure or suction. Collection traps also have been devised with the suction feature applied on a much larger scale than with the usual aspirator. Suction produced by a fan has been employed in traps in conjunction with light or other attractants. Some of these traps are described in the following references and in the section on Traps. Suction is created by a piston in a 'slurp-gun' described for aquatic collecting. This principle could be adapted for use in air to gather insects and to deposit them in a vial attached to the side of the piston.

References: Azrang 1976; Barnard & Mulla 1977;

Bradbury & Morrison 1975; Clifford et al. 1977; Evans et al. 1964; Galtsoff et al. 1937; Hurd 1954; Johnson 1950; Johnson & Taylor 1955; Johnson et al. 1957; Lumsden 1958; Minter 1961; Mulhern 1942; Scholdt & Neri 1974; Taylor 1962a; Turnbull & Nicholls 1966; White 1964; Weins & Burgess 1972; Williams 1973; Woke 1955.

1.7 Beating Sheets

A beating sheet should be made of durable cloth, preferably white, attached to a frame about 1 meter square, with two pieces of doweling or other light wood crossing each other and fitted into pockets at each corner of the cloth. An ordinary light-colored umbrella also may be used as a beating sheet. Place the beating sheet or umbrella under a tree or shrub and sharply beat the branches or foliage with a club or stick. Specimens will fall onto the sheet and may be removed from the light-colored material by hand or with forceps, a moistened brush, or an aspirator. Locating specimens on the sheet is sometimes a problem because of leaves or other unwanted material dropping onto the sheet. Watching for movement will help locate specimens, as well as tilting the sheet so that the debris is displaced or even allowed to fall off, with the insects and mites left clinging to the cloth.

Beating sheets are especially useful in collecting beetles, true bugs, and larval Lepidoptera. Beating may be the best collecting technique when the weather has turned cold, or early and late in the day, when normally active insects seek shelter in vegetation and are otherwise difficult to detect.

A ‘ground cloth’ also is used in sampling crop fields (see Rudd & Jensen 1977).

1.8 Sifters

Sifters are used to collect insects and mites that live in ground litter, leaf mold, rotting wood, mammal and bird nests, fungi, shore detritus, lichens, mosses, and similar material. Sifters are especially useful for winter collecting to pick up hibernating specimens. Almost any container with a wire-mesh screen bottom will serve as a sifter. The size of the mesh depends on the size of the specimens sought. For general purposes, screening with 2.5-3 meshes per centimeter is satisfactory. To use the sifter, place the material to be sifted into the container and shake it gently over a white pan or piece of white cloth. As the insects and mites fall onto the cloth, they may be collected with forceps, a brush, or an aspirator.

A similar method is used chiefly to collect mites from foliage. Using a sifter of 20-mesh screen (about 8 per centimeter) with a funnel underneath that leads to a small vial, beat pieces of vegetation against the screen to dislodge the mites, which will fall through the screen and into the vial below.

Another type of sifter employs two hoops of heavy metal, each with a handle. A long (3-4 ft.) canvas bag is sewn to the top hoop. The bag is left open at the end and secured with a cord or twist-tie. About 1 foot down in the bag, the second hoop is sewn to the canvas and to this is attached a metal screen. Coarse debris is loaded into the top and sifted down to the end of the canvas bag. Sifted debris is then ready to be processed by one of the following separators or extractors.

References: Martin 1977.

1.9 Separators and Extractors

Somewhat similar to the sifter are various devices designed to separate or extract live specimens from substances in which they may be found, such as leaf mold and other kinds of vegetable matter, shore detritus, dung, even net sweepings that include so much foreign matter that it is difficult to pick out the insects. These devices usually depend on some physical aid such as light, heat, or dryness to impel the insects to leave the foreign matter.

One of the simplest such devices is the sweeping separator (fig. 7). This is simply a carton or wooden box with a tight-fitting lid. Near the top of the box on one side is inserted a glass jar. If the jar is made with a screw top, a hole of proper diameter cut in the side of the carton will permit the jar to be screwed onto it. The cover ring,



Fig. 6. Separation bag

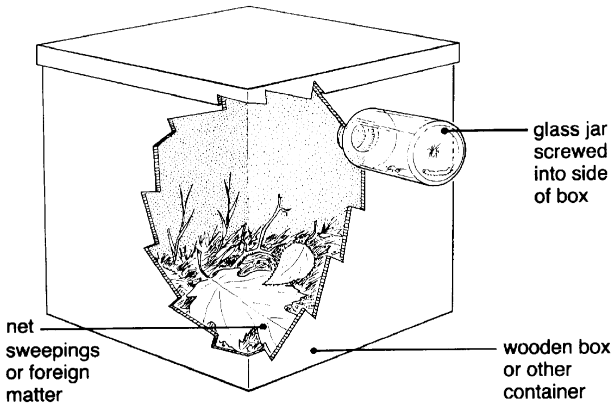


Fig. 7. Sweeping separator

without the lid, from a home-canning jar may be nailed to the periphery of a hole in a wooden box and the jar then screwed onto the ring.

The sweepings are dumped into the box and the cover is quickly closed. The insects in the darkened box soon will be attracted to the lighted glass jar. When all the insects appear to have entered the jar, it can be removed and its contents put into a killing jar. Alternatively, a jar cover containing a piece of blotting paper soaked with xylene may be placed over the jar for awhile to stun the insects, which may then be sorted.

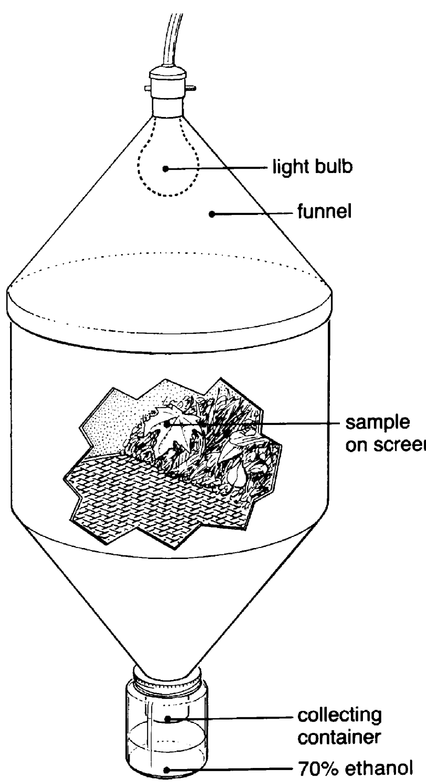


Fig. 8. Berlese funnel

A more sophisiticated version of this separtor is made of alumnium with a clear plastic top and cloth collecting bag (fig. 6). Sitting on three legs, this separator allow the collector to dump the catch into the bag, place the lid, which is lined with a magnet, on top and insert an aspirator through a small hole in the side of the bag. As insects are attracted to the top and collect on the plastic, the aspirator can be moved about to suck up the insects of interest.

Nets can also be modified to help keep plant material contacted during sweeping away from the insects. In most cases, hardware cloth or some other screening material with fairly large holes (ca. 1cm in diameter) is placed across the net opening and fastened to the net ring. This works well to keep out larger pieces of plant debris but will not be effective in excluding seeds and other small plant parts (Noyes 1988; Zolnerowich et al. 1990).

Insects collected into alcohol can also be separated from plant debris by the use of screens. In this method, a screen of 1/4 inch diameter galvanized hardware cloth is fastened over a frame. Below this is another screen made from a very fine mesh material such as organdy or a small section of panty hose. The insect/plant material collection is poured over the coarser screen and alcohol is added. When agitated, the insect will sink through the larger screen while plant material will float or be stopped by the screen mesh. A similar method uses a set of three stacked screens of decreasing diameter and specimens are washed from one layer to another using a gentle spray of water. Care must be taken so that the washing does not damage the specimens.

The Berlese or Tullgren funnel (Upton 1991) (fig. 8) and its modifications are cleaner and more efficient than sifting to separate insects and mites from leaf mold and similar materials. The sample (usually presifted to remove large debris) is placed on a screen near the top of a funnel. A light bulb can be placed above the sample to produce heat and light, which drive the insects downward into the funnel, or heated coils or a jacket around the funnel can be used to dry the sample and make it inhospitable. The insects and mites are directed by the funnel into a container, sometimes containing alcohol at the bottom of the funnel. Care should be taken not to dry the sample so rapidly that slow-moving specimens are immobilized before they can leave the sample. To prevent large amounts of debris from falling into the container, place the sample on the screen before the container is put in place.

A similar separator is the photoeclector or Winkler/Moczarski Elector. This device is similar to the Berlese funnel except that no light bulb or other heat source is used to drive the insect to the bottom. Instead, an open jar, with a most cloth or tissue inside, is attached to the bottom of the funnel or canvas bag and insects are

attracted to the light and humidity. An added advantage of this device is that it requires no electricity and so may be more readily used in the field.

References: Besuchet et al. 1987; Brown 1973; Everett & Lancaster 1968; Finch & Skinner 1974; Gui et al. 1942; Kempson et al. 1962; Kevan 1955; Kevan 1962; Lane & Anderson 1976; Martin 1977; Masner & Gibson 1979; Murphy 1962; Newell 1955; Norton and Kethley 1988; Salmon 1946.

1.10 Traps

Since a trap is defined as anything that impedes or stops the progress of an organism, this subject is extensive, including devices used with or without baits, lures, or other attractants. Besides its construction, the performance of a trap depends on such factors as its location, time of year or day, weather, temperature, and kind of attractant used, if any. A little ingenuity coupled with knowledge of the habits of the insects or mites sought will suggest modifications or improvements in nearly any trap or may even suggest new traps.

Only a few of the most useful traps are discussed here, but the following references describe many more, especially Martin 1977; Peterson 1964; Southwood 1979.

References: A'Brook 1973; Banks 1959; Banks et al. 1981; Barber 1931; Bidlingmayer 1967; Broadbent 1949; Broadbent et al. 1948; Dunn & Reeves 1980; Evans 1975; Flaschka & Floyd 1969; Ford 1973; Glasgow & Duffy 1961; Golmeric & Davenport 1971; Granger 1970; Hafraoui et al. 1980; Hanec & Bracken 1964; Hansens et al. 1971; Hargrove 1977; Hartstack et al. 1968; Hathaway 1981; Heathcote et al. 1969; Hienton 1974; Hollingsworth et al. 1963; Howell 1980; Kimerle & Anderson 1967; Klein et al. 1973; Martin 1977; Meyerdirk et al. 1979; Morris 1961; Peterson 1964; Pickens et al. 1972; Southwood 1979; Sparks et al. 1980; Taylor 1962b; Thorsteinson et al. 1965; Weseloh 1974; Whittaker 1952; Williams 1951; Woke 1955.

1.10.1 Effects of Elevation

One of the external factors affecting the performance of traps, especially light traps, has been specially studied, namely the effect of the elevation (above sea or ground level) at which the trap is placed when in use. The subject is complex, with many variables related to kinds of insects, locality, and so forth, which are discussed in the following references.

References: Blakeslee et al. 1959; Callahan et al. 1972; Cooke 1969; Frost 1957; Glick 1939; Glick 1957; Goma 1965; Meyers 1959; Roling & Kearby 1975; Stewart & Lam 1968.

1.10.2 Windowpane Traps

One of the simplest and cheapest traps is a barrier consisting of a windowpane held upright by stakes in the ground or suspended by a line from a tree or from a horizontal line. A trough filled with a liquid killing agent is so placed that insects flying into the pane drop into the trough and drown. They are removed from the liquid, washed with alcohol or other solvent, then preserved in alcohol or dried and pinned. The trap is not recommended for adult Lepidoptera or other insects that may be ruined if collected in fluid.

A modification of this trap uses the central "pane" of a malaise trap instead of a pane of glass. The malaise trap pane covers more space than glass, is easier to transport, and, of course, is not breakable. Various mesh sizes if cloth can also be used depending on the insects targeted. These traps may also be referred to as flight intercept traps.

References: Chapman & Kinghorn 1955; Corbet 1965; Kato et al. 1966; Lehker & Deay 1969; Masner and Goulet 1981; Nijholt & Chapman 1968; Peck and Davies 1980; Roling & Kearby 1975; Wilson 1969.

1.10.3 Interceptions Nets and Barriers

A piece of netting, 1.8 meters or more in height, can be stretched between three trees or poles to form a V-shaped trap with the wide end of the V open. A triangular roof should be adjusted to slope gently downward to the broad open side of the V. A device of this type will intercept many kinds of flying insects, particularly if the trap is situated with the point of the V toward the side of maximum light and in the direction of air movement. A pair of such nets set in opposite directions, or a single net in a zigzag shape, will intercept specimens from two directions. Since insects flying into such a net tend to gather at the pyramidal apex, they are easy to collect. In one variant of this trap the cloth is sprayed with a sythetic pyrethroid insecticide and the insects which are killed by contact with the cloth then fall into a long pan trap at the bottom. The so-called ‘funnel’ or ‘ramp’ traps are interception devices that direct insects to a central point, where a retaining device or killing jar may be placed. More complex arrangements have been described in the literature, primarily for migrating butterflies.

References: Gillies 1969; Graham et al. 1961; Hocking & Hudson 1974; Jonasson 1954; Leech 1955; Masner and Goulet 1981; Merrill & Skelly 1968; Nielsen 1960; Parman 1931, 1932; Steyskal 1981; Walker and Lenczewski 1989; Walker and Whitesell 1993, 1994.



Fig. 9. Malaise trap.

1.10.4 Malaise Traps

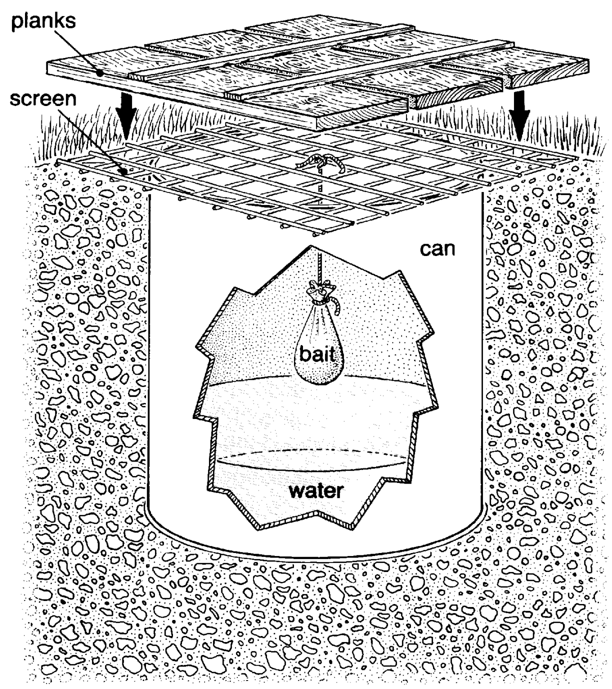
One of the most widely used insect traps was developed by the Swedish entomologist René Malaise and that now bears his name. Several modifications of his original design have been published, and at least one is available commercially. The trap, as originally designed, consists of a vertical net serving as a baffle, end nets, and a sloping canopy leading up to a collecting device (fig. 9). The collecting device may be a jar with either a solid or evaporating killing agent or a liquid in which the insects drown. The original design is unidirectional or bidirectional with the baffle in the middle, but more recent types include a nondirectional type with cross baffles and with the collecting device in the center. Malaise traps have been phenomenally successful, sometimes collecting large numbers of species that could not be obtained otherwise. Attractants may be used to increase the efficiency of the traps for special purposes.

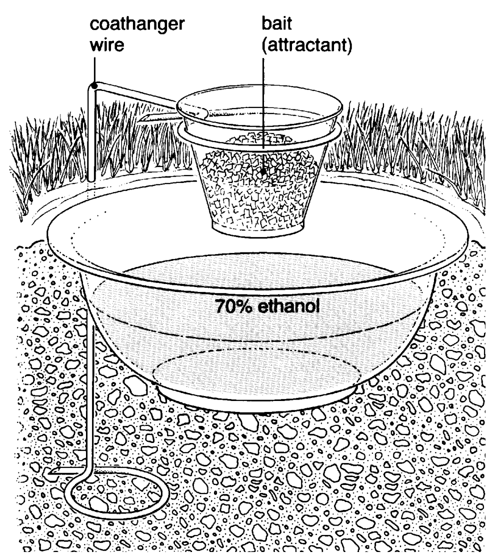
References: Butler 1966; Townes 1972; Steyskal 1981 (bibliography).

1.10.5 Pitfall and Dish Traps

Another simple but very effective and useful type of interception trap consists of a jar, can, or dish sunk in the earth (fig. 10). A cover must be placed over the open top of the jar to exclude rain and small vertebrates while allowing insects and mites to enter. A piece of bark, wood, or flat stone will serve this purpose. Pitfall traps may be baited with various substances, depending on the kind of insects or mites the collector hopes to capture. Although most that fall into the trap will remain there, it should be inspected daily, if possible, and desired specimens removed and placed in alcohol or in a killing bottle while they are in their best condition.

Also in the pitfall category is the cereal dish trap, which is a simple but effective device for obtaining insects attracted to dung. It consists of a small dish, preferably





Figs. 10-11. 10, Pitfall Trap (Top). 11, Cereal Dish Trap (Bottom).

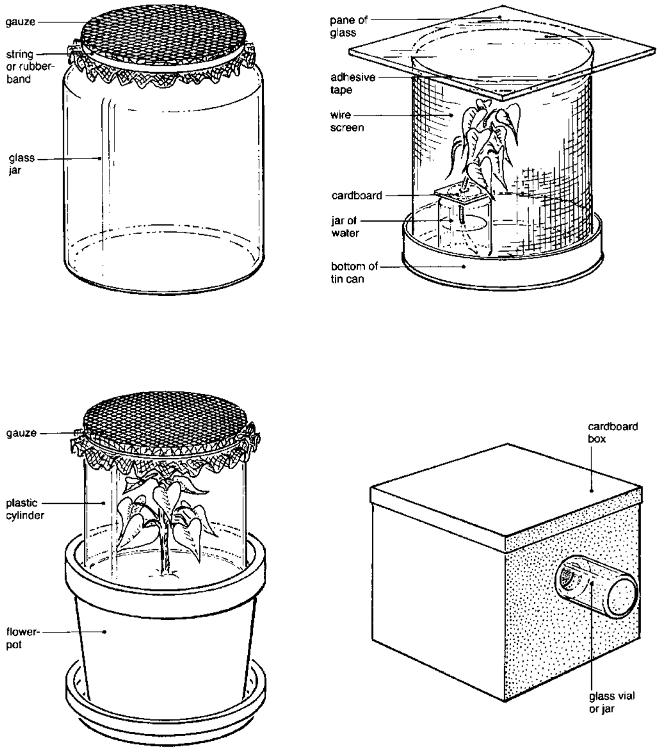


Fig. 12. Emergence and rearing traps.

with a rim, set in the earth (fig. 11) and partly filled with 70 percent ethanol, or, if available, with ethylene glycol, which does not evaporate. A piece of stout wire, such as a coathanger, is bent as shown, with a loop at one end to hold the bait receptacle. A few zigzag bends in the other end of the wire will keep the looped end from swinging after the wire is pushed into the earth. The bait receptacle may be a small plastic or metal cup such as is often used for medicine doses, or a coffee creamer, or a cup formed from aluminum foil. When baited with animal or human feces, this trap attracts beetles, mostly of the families Scarabaeidae and Staphylinidae, springtails, ants, earwigs, some parasitic Hymenoptera, and, rather surprisingly, several families of flies, especially Phoridae, Sepsidae, and Muscidae. The larger, strong-flying calliphorid and sarcophagid flies seldom fall into the liquid, although they are attracted to the bait. The alcohol fumes probably cause the smaller flies to drop into it. The trap is made of easily obtained materials, is easily transported, and provides excellent results. It deserves wide use.

References: Adlerz 1971; Barber 1931; Beaudry 1954; Briggs 1971; Clark and Blom 1992; Dethier 1955; Fichter 1941; Gist & Crossley 1973; Golding 1941; Greenslade 1973; Greenslade & Greenslade 1971; Greenslade 1964; Gressitt et al. 1961; Grigarick 1959; Heathcote 1957; Houseweart et al. 1979; Joosse 1975; Loschiavo 1974; Luff 1968, 1975; Masner & Huggert 1979; Morrill 1975 (bibliography); Muma 1975; Newton & Peck 1975; Reeves 1980; Schmid et al. 1973; Shubeck 1976; Smith 1976; Smith et al. 1977; Thomas & Sleeper 1977; Tretzel 1955; Van den Berghe 1992; Welch 1964.

1.10.6 Moericke Traps and Other Color Traps

Moericke traps or yellow pan traps are used extensively by some collectors. An aluminum or plastic pan is painted yellow and placed on the ground (or a depression may be dug and the pan set in the depression) and filled about 1/3 full with salt water, or some other non-toxic fluid. A few drops of detergent of some other surfactant is added to the water to break the surface tension. Insects attracted to the pan fall into the fluid and perish. The trap is then strained periodically (one favorite strainer is a small aquarium fish net). Pan traps such as this, are often placed under malaise traps and flight interception traps to catch insects that may hit the trap and fall to the ground.

Yellow seems to be the best color for traps, but various kinds of insects react differently to different colors. Some recent research indicates that certain parasitic wasps respond most strongly to blue.

Colored sticky traps are also used to sample insects in various habitats. One of these, the Manitoba trap (fig. 15) has a black sphere to attract horse flies (family Tabanidae), which are then captured in a canopy-type trap.

References: Beroza 1972; Granger 1970; Gurney et al. 1964; Hottes 1951; Kieckhefer et al. 1976; Kring 1970; Marshall, 1994; Moericke 1951, 1955 (in german); Prokopy 1973; Weseloh, 1986.

1.10.7 Emergence and Rearing Traps

An emergence trap is any device that prevents adult insects from dispersing when they emerge from their immature stages in any substrate, such as soil, plant tissue, or water. A simple canopy over an area of soil, over a plant infested with larvae, or over a section of stream or other water area containing immature stages of midges, mayflies, and other arthropods will secure the emerging adults. If it is equipped with a retaining device, as in the Malaise trap, the adults can be killed and preserved shortly after emergence. It must be remembered, however, that many insects should not be killed too soon after emergence because the adults are often teneral or soft bodied and incompletely pigmented and must be kept alive until the body and wings completely harden and colors develop fully. Emergence traps and rearing cages (fig. 12) enable

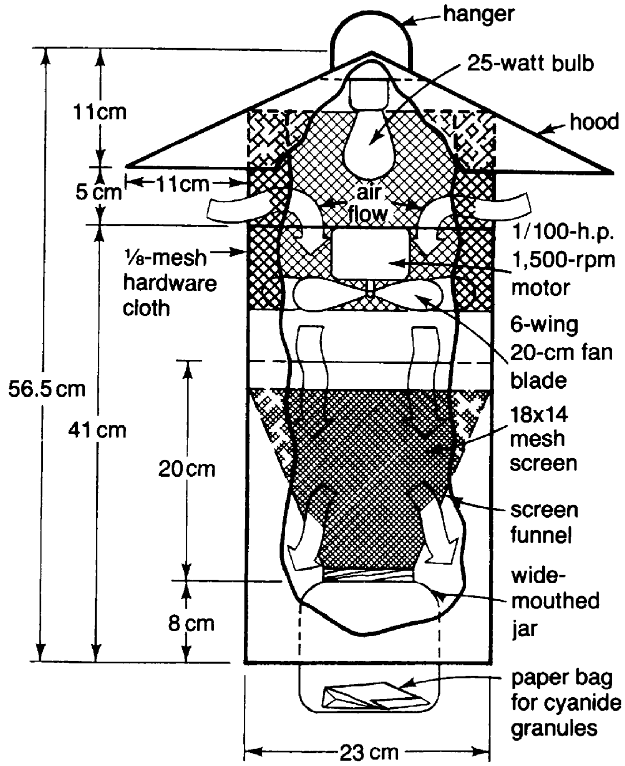


Fig. 13. New Jersey Trap

the insects to develop naturally while insuring their capture when they mature or when larvae emerge to pupate.

References: Adkins 1972; Akar and Osgood 1987; Banks et al. 1981; Barber & Mathews 1979; Butler 1966; Catts 1970; Cheng 1975; Coon & Pepper 1968; Davidson & Swan 1933; Debolt et al. 1975; Doane 1961; Gerking 1957; Glen 1976; Harwood & Areekul 1957; Hollis 1980; Kimerle & Anderson 1967; Krombein 1967; LaGasa & Smith 1978; Lammers 1977; Langford & Daffern 1975; Levin 1957; Lindeberg 1958; Macan 1964; McCauley 1976; Masteller 1977; Merritt & Poorbaugh 1975; Morgan et al. 1963; Morrill & Whitcomb 1972; Mundie 1956, 1964, 1966, 1971; Murray & Charles 1975; Needham 1937; Nielson 1974; Smith et al. 1977; Thompson & Gregg 1974; Turnock 1957; Yates 1974.

1.10.8 Lobster or Eel Trap

This category includes any container that has its open end fitted with a truncated cone directed inward, as in a lobster or eel trap, known as a ‘Reuse’ in German. An ordinary killing jar with a funnel fastened into its open end is an example. When the funnel is placed over an insect, the specimen will usually crawl or fly toward the light and enter the jar through the funnel. Modified traps of this type include the Steiner and McPhail traps, which are used primarily in fruit fly surveys but are suitable for many other purposes. The inside of the Steiner trap usually has a sticky material containing a pheromone or other lure. Both traps, as well as similar devices, may be used with different attractants to collect diverse kinds of insects.

References: Bellamy & Reeves 1952; Broce et al. 1977; Brockway et al. 1962; Doane 1961; Hollis 1980; Jacobson & Beroza 1964; Morrill & Whitcomb 1972; Nakagawa et al. 1975; Nicholls 1960; Nielson 1974; Reierson & Wagner 1975; Steyskal 1977.

1.10.9 Light Traps

With light traps, advantage is taken of the attraction of many insects to a light source. Using various wave-lengths as the attractant, a great variety of traps can be devised, a few of which are described here.

Many traps can be constructed easily from materials generally available around the home. All wiring and electrical connections should be approved for outdoor use. Funnels can be made of metal, plastic, or heavy paper. Traps can be used with or without a cover, but if they are to be operated for several nights, covers should be installed to keep out rain.

The New Jersey trap (fig. 13) includes a motorized fan to force insects attracted to the light into a killing jar. It has been especially useful for collecting small, non-scaly insects such as midges and gnats. This type of light trap, in which the insects fall directly into a killing jar, is not recommended for use with moths because such delicate

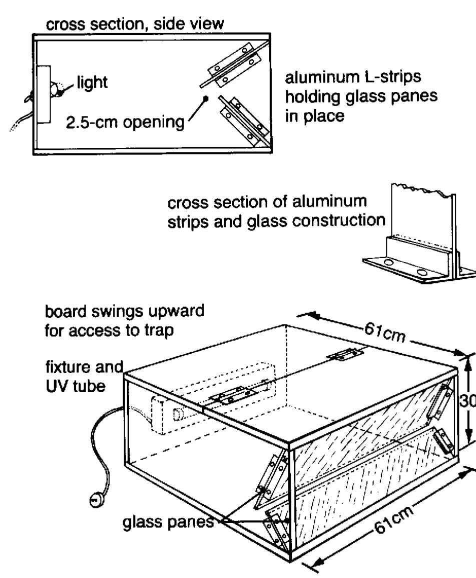


Fig. 14. Wilkinson Trap

specimens may be badly rubbed or torn. If only small insects are desired, they may be protected from damage by larger insects by placing a screen with the proper sized mesh over the entrance. The Minnesota trap is very similar to the New Jersey trap, but it does not include a fan or any motorized method of draft induction.

The Wilkinson trap (fig. 14) requires somewhat more effort to construct than the preceding traps, but it has the advantage of confining, not killing, the trapped insects. Moths, therefore, can be collected in good condition if the trap is inspected frequently and desirable specimens are removed quickly through the hinged top and placed in a killing jar.

Several highly effective but more elaborate devices have been made for collecting moths and other fragile insects in good condition. Basically, they all use the principle of a funnel with a central light source above it and vanes or baffles to intercept the approaching insects that are dropped through the funnel into the container beneath, which may or may not hold a killing agent. The nature of the container and the type of killing agent affect the quality of the specimens obtained. Some traps catch the insects alive in a large collection chamber, such as a garbage can, which is filled or nearly filled with loosely arranged egg cartons. Most moths will come to rest in the cavities between the egg cartons and will remain there until removed in the morning.

Other traps are designed to kill the insects by means of high concentrations of fumes from a liquid killing agent, such as tetrachloroethane or calcium cyanide. A heaping tablespoon or more of calcium cyanide is placed in each of four to six brown paper bags, which are hung in a large garbage can or other large container. A dampened cloth, such as a washcloth, is also hung inside the can to humidify the air and activate the cyanide. This is especially necessary in dry weather. The concentration of the gas inside the can is so great that insects are inactivated almost instantly on entering, and even the most delicate specimens are damaged very little. The bags containing the calcium cyanide powder should be replaced as needed. If two of the oldest bags are replaced with two fresh ones each successive night, the trap can be run as long as the collector desires.

Handle cyanide outdoors, facing downwind, and with extreme caution. During the day, when the trap is not in use, store the cyanide bags in an airtight container. All forms of cyanide used as killing agents react and break down quickly when exposed to air and moisture; nevertheless dispose of the residue carefully.

To prevent rainwater from accumulating in the trap, place a screen-covered funnel inside the collection chamber to drain the water out through a hole in the bottom of the trap. Sometimes a system of separators is added to guide beetles and other heavy, hard-bodied insects into a different part of the container than the moths and other delicate specimens.

The most efficient light traps use lamps rich in their output of ultraviolet light. The British-made Robinson trap employs an intense, blue-white, 125-watt mercury vapor lamp of a type used for street lighting. This, the most effective insect attractant commercially marketed, is widely used in many kinds of light traps because it has some special advantages over other kinds of attractants. For example, this type of lamp is the only one that emits the kind of light that attracts large numbers of *Catocala* (underwing) moths, a colorful group popular with many collectors.

Many traps are equipped with 15-watt ultraviolet fluorescent tubes, which emit a highly visible bluish-white light, although blacklight tubes emitting deep purple light are similarly effective. Ultraviolet tubes of lower or higher wattage also may be used and are all highly effective. A 15-watt ultraviolet tube has been estimated to attract about 10 times as many insects as a 500 candlepower gasoline lantern or incandescent lamp. The advantage of the fluorescent tube over the mercury vapor lamp is that it is less expensive and much more portable. A 15-watt tube is easily powered by an ordinary automobile battery by using an inverter to change 6- or 12-volt direct current to 120-volt alternating current. Also, its ultraviolet output is not strong enough to cause any significant eye damage. The safety factor of the mercury vapor lamp at close range is less certain, although entomologists who have used the Robinson trap for many years seem to have suffered no ill effects.

A new, lightweight, spillproof 12-volt battery, in which the acid electrolyte is a gel rather than a liquid, is far superior to the standard automotive battery for powering light traps, but it is fairly expensive and requires a special charger. Special lightweight, nickel-cadmium battery packs, used to power blacklights for collecting, are marketed by some dealers of entomological equipment.

1.10.10 Light Sheets

Another highly effective method of using light to attract moths and other nocturnal insects is with a light sheet (fig. 15). This is simply a cloth sheet, usually a white bedsheet, hung outdoors at night with an appropriate light source or combination of sources such as ultraviolet fluorescent tubes, gasoline lanterns, or automobile headlights placed a few feet in front of it. As insects are attracted and alight on the sheet, they are easily captured in cyanide bottles or jars by the collector who stands in attendance or at least checks the sheet frequently. The



Fig. 15. A light sheet in the field

sheet may be pinned to a rope tied between two trees or fastened to the side of a building, with the bottom edge spread out on the ground beneath the light. Some collectors use supports to hold the bottom edge of the sheet several centimeters above the ground so that no specimens can crawl into the vegetation under the sheet and be overlooked. Other collectors turn up the edge to form a trough into which insects may fall as they strike the sheet.

The light sheet remains unsurpassed as a method of collecting moths in flawless condition or of obtaining live females for rearing purposes. Its main disadvantage is that species that fly very late or those that are active only in the early morning hours may be missed unless one is prepared to spend most of the night at the sheet. Many other insects besides moths are attracted to the sheet, and collectors of beetles, flies and other kinds of insects would do well to collect with this method.

It should be emphasized that the phases of the moon may influence the attraction of insects to artificial light. A bright moon may compete with the light source resulting in a reduced catch. The best collecting period each month extends from the fifth night after the full moon until about a week before the next full moon.

References (light traps and sheets): Andreyev et al. 1970; Apperson & Yows 1976; Barr et al. 1963; Barrett et al. 1971; Bartnett & Stephenson 1968; Belton & Kempster 1963; Belton & Pucat 1967; Blakeslee et al. 1959; Breyev 1963; Burbutis & Stewart 1979; Carlson 1971; Carlson 1972; Clark & Curtis 1973; Davis & Landis 1949; DeFoliart 1972; Freeman 1972; Frost 1952, 1964; Graham et al. 1961; Gurney et al. 1964; Hardwick 1968; Hathaway 1981; Hollingsworth & Hartstack 1972; Hollingsworth et al.; Howell 1980; Kovrov & Monchadskii 1963; Lowe & Putnam 1964; McDonald 1970; Meyers 1959; Miller et al. 1970; Morgan & Uebel 1974; Mulhern 1942; Nantung Institute of Agriculture 1975; Onsager 1976; Powers 1969; Pratt 1944; Smith et al. 1974; Stanley & Dominick 1970; Stewart & Payne 1971; Stewart & Lam 1968; Tedders & Edwards 1972; USDA 1961; White 1964; Wilkinson 1969; Williams 1948; Zimmerman 1978.

1.10.11 Sticky Traps

In this type of trap, a board, piece of tape, pane of glass, piece of wire net, cylinder, or other object, often painted yellow, is coated with a sticky substance and suspended from a tree branch or other convenient object. Insects landing on the sticky surface are unable to extricate themselves. The sticky material is later dissolved with a suitable solvent, usually toluene, xylene, ethylacetate, or various combinations of these, and the insects are washed first in Cellosolve and then in xylene. This type of trap should not be used to collect certain specimens, such as Lepidoptera, which are ruined by the sticky substance and cannot be removed without being destroyed.

Various sticky-trap materials are available commercially, some with added attractants. However, use caution in selecting a sticky substance because some are difficult to dissolve.

References: Buriff 1973; Chiang 1973; Dominick 1972; Edmunds et al. 1976; Evans 1975; Gillies & Snow 1967; Golding 1941, 1946; Goodenough & Snow 1973; Harris et al. 1971; Harris & McCafferty 1977; Heathcote 1957; Johnson 1950; Lambert & Franklin 1967; Mason & Sublette 1971; Maxwell 1965; Moreland 1955; Murphy 1962 (pp.226-227), 1985; Prokopy 1968; Still 1960; Taylor 1962b; Williams 1973.

1.10.12 Snap Traps

Two kinds of traps designed for quantitative sampling may be termed “snap traps.” One of them (see Menzies & Hagley 1977) consists of a pair of wooden or plastic discs, slotted to the center so as to fit on a tree branch and connected to each other by a pair of rods. A cloth cylinder is affixed at one end to one of the discs and at the other end to a ring sliding on the rods. After the cloth cylinder has been pulled to one end and has been secured in place, the ring is held by a pair of latches. When insects have settled on the branch, its leaves, or flowers, the latches are released by pulling on a string from a distance, and the trap is snapped shut by a pair of springs on the rods, capturing any insects present. One of the canopy traps (see Turnbull & Nichols 1966) operates in a similar fashion. When a remotely controlled latch is

pulled, a spring-loaded canopy is snapped over an area of soil, and insects within the canopy are collected by suction or a vacuum device. This trap was designed for use in grasslands.

1.10.13 Artificial Refuges

Many insects, especially beetles, are successfully found under stones, planks, or rotten logs. Providing such refuges, as pieces of wood, card board, or even complex traps, is also a form of trapping. Lepidoptera larvae, for example, will congregate under burlap tied in a band around the trunks of trees. This technique has even been used to help control some pest species such as the gypsy moth.

References: Campion 1972; Shubeck 1976.

1.10.14 Electrical Grid Traps

In recent years, electrocuting pest insects has been used extensively in control work. The insects are attracted to a device by a pheromone or other lure placed in a chamber protected by a strongly charged electrical grid. The method deserves study for other purposes, such as surveying the arthropod fauna of an area.

References: Goodenough & Snow 1973; Hartstack et al. 1968; Mitchell et al. 1972, 1973, 1974; Rogers & Smith 1977; Stanley et al. 1977.

1.11 Baits, Lures, and Other Attractants

Any substance that attracts insects may be used as a bait. Natural products, chemicals derived therefrom or synthesized, and secretions of the insects themselves may all be used as attractants. Mere exposure of the substance may be considered as setting up a trap, and attractive substances are used in many constructed traps.

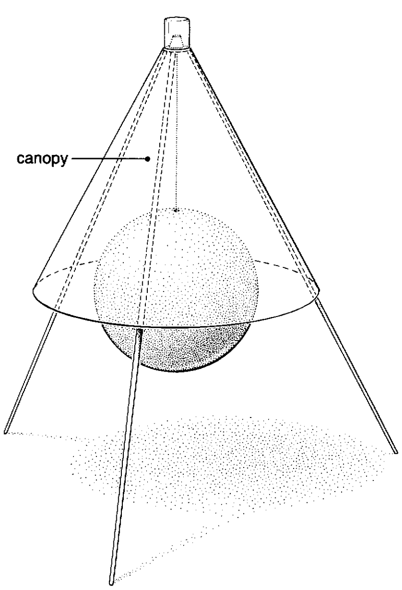


Fig. 15. A Manitoba trap.

Sugaring for moths, one of the oldest collecting methods, involves the use of a specially prepared bait in which some form of sugar is an essential component.

The bait may be refined or brown sugar, molasses, or sirup. Such substances often are mixed with stale beer, fermented peaches, bananas, or some other fruit— there is no standard formula. Each lepidopterist has his or her own favorite recipe.

One particularly satisfactory recipe uses fresh, ripe peaches; culls or windfalls are suitable. Remove the seeds but not the skins, mash the fruit, then place it in a 4-liter (1-gal) or larger container of plastic, glass, stainless steel, enamelware, or crockery with a snugly fitting but not tight cover. Avoid using metal containers that may rust or corrode. Fill each container only onehalf to two-thirds full to allow space for expansion. Add about a cup of sugar and place in a moderately warm place for the mixture to ferment. The bubbling fermentation reaction should start in a day or so and may continue for 2 weeks or more, depending on the temperature. During this time, check the fermentation every day or every other day and add sugar until fermentation appears to have subsided completely. As the added sugar is converted to alcohol, the growth of yeast slows and eventually ceases.

After fermentation ceases, the bait should remain stable and should then be kept in tightly sealed containers to prevent contamination and evaporation. If the mixture is allowed to run low in sugar during the fermentation process, vinegar will be produced instead of alcohol. It is therefore important to smell the bait periodically and to add plenty of sugar to avoid this. The amount of sugar consumed will be surprising, usually over 0.4 kg per liter (3.3 lb per gal). The bait should have a sweet, fruity, winelike fragrance. A trace of vinegar is not objectionable but is better avoided. Canned fruit, such as applesauce, may also be used to make the bait, but inasmuch as such products are completely sterile, a small amount of yeast must be added to start fermentation. Although the bait may seem troublesome to prepare, it keeps for years and is thus available at any time, even when fruit is not in season.

Immediately before use, the bait may be mixed with 30 to 50 percent molasses or brown sugar or a mixture of these. This thickens the bait so that it will not dry out so quickly, and it makes the supply last longer.

The best time to set out the sugar bait is in the early evening before dark. It may be applied with a paint brush

in streaks on tree trunks, fenceposts, or other surfaces. Choose a definite route, such as along a trail or along the edge of a field, so that later you can follow it in the dark with a lantern or flashlight. Experienced collectors learn to approach the patches of bait stealthily with a light in one hand and a killing jar in the other to catch the moths before they are frightened off. Some collectors prefer to wear a headlamp, leaving both hands free. Although some moths will fly away and be lost, a net usually is regarded as an unnecessary encumbrance, because moths can be directed rather easily into the jar. Sugaring is an especially useful way to collect noctuid moths, and the bait applied in the evening often will attract various diurnal insects on the following days. The peach bait previously described has been used in butterfly traps with spectacular results. However, collecting with baits is notoriously unpredictable, being extremely productive on one occasion and disappointing on another, under apparently identical conditions.

1.11.1 Baiting With Feces.

Animal and human feces attract many insects. A simple but effective method of collecting such insects is to place fresh feces on a piece of paper on the ground and wait a few minutes. When a sufficient number of insects have arrived, a net with its bag held upward can be brought carefully over the bait about 1 meter above it. This will not disturb the insects, nor will they be greatly disturbed when the net is lowered gently about two- thirds of the distance to the bait. At this point, the net should be quickly lowered until its rim strikes the paper. The insects, mostly flies, will rise into the net, which may then be lifted a short distance above the bait and quickly swung sideways, capturing the insects in the bottom of the bag. In about half an hour, many flies can be caught, virtually all that have come to the bait. Because of this, the ‘baiting with feces’ method may be used for quantitative studies (see Steyskal 1957).

Feces are most attractive to insects during the first hour after deposition, but insects coming for a more extended period may be captured by placing a canopy trap over the feces or by using the feces with the cereal dish trap (see p. 12). Emergence traps placed over old feces will capture adult insects emerging from immature forms feeding there. The same methods also may be used with other baits, such as decaying fruit, small carcasses, and a wide variety of other substances.

1.11.2 The Oatmeal Trail

Hubbell (1956) showed that dry oatmeal scattered along a path will attract such insects as crickets, camel crickets, cockroaches, and ants. Some of these insects feed only at night and may be hand-collected by flashlight or by light from a headlamp. A killing bottle is used, and the specimens are collected with fingers, an aspirator, or a net.

1.11.3 Pheromones and Other Attractants

Substances naturally produced by insects to attract others of their own kind are known as pheromones. They are often used in traps to aid in controlling pest species. Most pheromones are highly specific, attracting only one species or a group of closely related species. “Spanish Fly” (cantharidin) has recently come into use as an extremely effective attractant for various beetles, such as pedilids, and bugs, such as bryocerines. Female specimens of certain insects, such as cicadas and silkworm moths, may be placed alive in a trap and used as a bait with their pheromones and the sounds they produce attracting males. Female saturniids (silkworm moth) may be used to attract males which may come from great distances. The pheromones of sesiid moths are commercially available and can be attached to the collector's net or hung over a dish with ethylene glycol.

Host animals likewise may be used as bait for various bloodsucking insects, with or without constructed traps. Carbon dioxide in the form of “Dry Ice,” cylinder gas, or marble chips treated with an acid such as vinegar serves as an attractant for certain insects and has been very successful in attracting horse flies to Malaise and Manitoba traps.

1.11.4 - Sounds, etc.

Sounds are produced by many insects to attract others of their own kind. These sounds are very specific in pitch, tempo, and duration. Recordings of such sounds, played at the proper volume, have been effective in luring grasshoppers, crickets, and other kinds of insects.

Hesperiid moths (skippers) have been shown to be attracted to small pieces of wetted paper placed on vegetation (Lamas et al., 1993).

References for attractants: General—Acree et al. 1968; Atkins 1957; Beavers et al 1972; Beroza 1970, 1972; Beroza & Green 1963; Bram 1978; Carestia & Savage 1967; Clinch 1971; Coffey 1966; Debolt et al. 1975; DeJong 1967; Fahy 1972; Golding 1941; Greenslade 1964; Hocking 1963; Howell 1980; Hubbell 1956; Jacobson & Beroza 1964; Laird 1981; Lee et al. 1982; LeSage & Harrison 1979; Luff 1975; Macleod & Donnelly 1956; Mason 1963; Morris & DeFoliart 1969; Nakagawa et al. 1971; Newhouse et al. 1966; Pinniger 1975; Rennison & Robertson 1959; Roberts 1972; Sanders & Dobson 1966; Shorey & McKelvey 1977; Steyskal 1957; Strenzke 1966; Walsh 1933; Wellso & Fischer 1971; Wilton 1963; carbon dioxide—Blume et al. 1972; Carestis

& Savage 1967; Davidson & Swan 1933; Debolt et al. 1975; Evans 1975; Fahy 1972; Gillies & Snow 1967; Hoy 1970; Kato et al. 1966; Knox & Hays 1972; Morris & DeFoliart 1969; Newhouse et al. 1966; Reeves 1951, 1953; Rennison & Robertson 1959; Roberts 1972; Snoddy & Hays 1966; Stryker & Young 1970; Takeda et al. 1962; Whitsel & Schoeppner 1965; Wilson et al. 1972; pheromones—Beaudry 1954; Bellamy & Reeves 1952; Beroza et al. 1974; Birch 1974; Campion 1972; Campion et al. 1974; Goonewardene et al. 1973; Hathaway 1981; Holbrook & Beroza 1960; Howell 1980; Howland et al 1969; Jacobson 1972; Jacobson & Beroza 1964; Mitchell et al. 1972; Neal 1979; Peacock & Cuthbert 1975; Shorey 1973; Shorey & McKelvey 1977; Sparks et al. 1980; Steck & Bailey 1978; Weatherston 1976; sound—Belton 1962; Cade 1975.

1.12 - Collecting Aquatic and Soil Insects and Ectoparasites

Insects and mites emerging from water may be collected by the same means as terrestrial insects, but specialized equipment is required. Aquatic insects are of great importance to water quality and are being intensely investigated in biodiversity studies. The following references pertain to aquatic collecting.

References: Apperson & Yows 1976; Carlson 1971; Coon & Pepper 1968; Coulson et al. 1970; Eastop 1955; Edmondson & Winberg 1971; Edwards et al. 1981; English 1987; Essig 1958; Gerking 1957; Hodgson 1940; Jonasson 1954; Kimerle & Anderson 1967; LaGasa & Smith 1978; Langford & Daffern 1975; Lawson & Merritt 1979; LeSage & Harrison 1979; Macan 1964; McCauley 1976; Mason & Sublette 1971; Masteller 1977; Merritt et al. 1978 (general); Morgan et al. 1963; Mundie 1956, 1964, 1966, 1971; Murray & Charles 1975; Pennak 1978 (general); Piecrynski 1961; Sladeckova 1962; Tarshis 1968a, 1968b; Waters 196; Welch 1848 (general); Wood & Davies 1966; Weber 1987; Wood et al. 1979.

As with aquatic specimens, insects and mites that live on or under the soil surface require special techniques and equipment for their collection and study. Many soilinhabiting species are of great economic importance because they devour the roots of crops. Many spend their immature stages in soil but emerge and leave the soil as adults. A considerable amount of literature on soil insects has been published, the most useful of which is cited here. See also the references cited under Separators and Extractors (p. 9) and Pitfall and Dish Traps (p. 12)

References: Barnes 1941; Briggs 1971; Brindle 1963; Dethier 1955; Fessenden 1949; Kevan 1955, 1962; Kuhnelt et al. 1976; Lane & Anderson 1976; MacFayden 1962; Murphy 1962; Newell 1955; Paquin and Coderre 1996; Salt & Hollick 1944; Teskey 1962.

Some ectoparasites, particularly those that fly, may be collected in some of the traps discussed,using their hosts as bait; others may be collected by means of the special devices described in the following references.

References: British Museum 1974 (p. 152), Comstock 1940; Watson & Amerson 1967; Williamson 1954.

1.13 - Rearing

Collectors should take every opportunity to rear insects and mites. Not only are reared specimens generally in the best possible condition, but rearing provides life stages that otherwise might be collected only rarely or with great difficulty. By preserving one or more specimens from each of the stages as they are reared, if sufficient material is available, the collector can obtain series of immature stages along with associated adults. Such series are extremely desirable, especially for species in which the adult is known but the immature stages are unknown or difficult to identify. The converse often is true also—some species of insects, such as stem-mining flies, are fairly abundant in the larval stage but have never been reared to the adult stage; consequently, one does not know whether they are stages of a species that has been described and named from an adult but whose life history is unknown. Since adults of these flies are seldom found, the easiest way to obtain the stage necessary for specific determination is to rear the larvae or pupae.

If only a few specimens are reared, the shed skins and pupal cases or puparia should be preserved, as they are of value if properly associated with the reared adult. Do not preserve a pupa or puparium with an adult unless you are positive that the association is correct. It is best to put pupae in separate containers so that adults or parasites that emerge are associated with certainty. If at all feasible, the parasite's host should be preserved for identification. Keep careful notes throughout the rearing so that all data relative to the biology of the species are properly correlated.

1.13.1- Containers for Rearing

To rear specimens successfully, simulate as closely as possible in the rearing cages the natural conditions under which the immatures werq found. Almost any container will serve as a temporary cage for living insects or mites. One simple temporary cage that is very handy on field trips is a paper bag. Plant material or a soil sample containing insects or mites is placed in the paper bag, which is then sealed. A paper bag also can be placed over the top of a plant on which insects or mites are found. The

bottom edge of the bag is tied tightly around the exposed stems, which are cut and placed in a jar of water. One disadvantage of using a paper bag is that it is not transparent, so it must be removed to observe the specimens or to determine when the foliage needs to be changed. Clear plastic bags are better suited to such viewing. Plastic bags with a paper towel placed in the bottom are extrememly efficient rearing containers for leaf mining and other small moths. They require frequent inversion to minimize condensation.

Another simple temporary cage is a glass jar with its lid replaced by a piece of organdy cloth or gauze held in place by a rubberband. A few such jars in a collecting kit are useful for holding live insects. For aquatic species, using a watertight lid on the jars is advisable. If aquatic insects are to be transported over a considerable distance, fewer will die if the jar is packed with wet moss or leaves than if the specimens are allowed to slosh around in water alone. After arrival at your destination, release the insects into a good rearing container (fig. 12).

Aquatic insects can be reared in their natural habitat by confining them in a wire screen or gauze cage, part of which is submerged in water. Be sure to anchor the cage securely. The screen used in aquatic cages should be coarse enough to allow food to flow through, yet fine enough to retain the insects being reared. Certain aquatic insects may be reared readily indoors in an aquarium or even in a glass jar. The main goal is to try to duplicate their natural habitat. If the specimen was collected from a rapidly flowing stream, it is unlikely to survive indoors unless the water is aerated. Other insects do well in stagnant water. Aquatic vegetation usually should be provided in the aquarium even for predaceous specimens, such as dragonfly nymphs, which often are found clinging to underwater stems. Keep sufficient space, which will vary according to the insect being reared, between the surface of the water and the screen or gauze cover over the aquarium to allow the adult insect to emerge. A dragonfly, for example, needs considerable space, plus a stick, rock, or other object on which to perch after emerging so that the wings will develop fully.

Most adult insects, both terrestrial and aquatic, are teneral when they first emerge and should not be killed until the exoskeleton and wings harden and the colors develop fully. This may be a matter of minutes, hours, or even days. It is advisable to keep even small flies alive for 1 full day after they emerge. Specimens killed while still teneral will shrivel when mounted. Some insects, if kept in cages too long after emerging, especially butterflies and moths, will beat their wings against the cage and lose many scales or tear their wings. Providing adequate space in which emerging insects may expand their wings fully and move about slightly is therefore critical in the design of rearing cages.

Beetles and other boring insects often are abundant in bark and wood. If pieces of such material are placed in glass or metal containers, excellent specimens of the adults may be obtained, although sometimes not for a considerable time. Cages made of wood or cardboard are not suitable for such insects because those found in wood or bark usually are well equipped, both in immature and adult stages, to chew their way through a cage made of such material and thus escape.

A flowerpot cage is one of the best containers for rearing plant-feeding species over an extended period. The host plant, if its size and habitat permit, is placed in a flowerpot, and a cylinder of glass, plastic, or wire screen is placed around the plant (fig. 12, lower left).

Another type of flowerpot cage is made by inserting a cane or stick, taller than the plant, into the soil in the pot. One end of a net or muslin tube is fitted over the edge of the pot and is held in place by a string. The other end of the tube is tied around the top of the stick. An advantage of the flowerpot cage is that the plant is living, and fresh plant material need not be added daily.

Plant-feeding mites will not wander far as long as suitable host material is available for them. Because mites are wingless even as adults, they can be confined in an open rearing container by making a barrier around the top edge or upper inner sides of the container with Vaseline or talcum powder.

Emergence cages are essentially rearing cages that are used when it is impractical or impossible to bring specimens indoors. Emergence cages may also be considered as traps and are discussed under that heading (see p. 13). With plant- feeding insects, a sleeve consisting of a muslin tube with open ends is slipped over a branch or plant and tied at one end. The insects are then placed in the tube, and the loose end of the tube is tied. This cloth tube can be modified to allow observation of the insects by replacing the midsection with a “window” of clear plastic or wire screen. If the insects in the tube require duff or debris in which to pupate, the tube should be placed perpendicular to the ground and duff or debris placed in the lower end.

1.13.2 - Rearing Conditions and Problems

1.13.2.1 - Moisture

The moisture requirements of insects and mites are varied. Examination of the habitat from which specimens were collected should provide clues about their moisture requirements in captivity. Many insects in the pupal stage are resistant to drought. Species that normally infest stored

foods also require very little moisture; in fact, many produce their own water. Most species found outdoors require higher levels of humidity than are generally found indoors. Additional moisture can be added to indoor rearing cages in several ways. To increase the humidity in a cage, keep a moist pad of cotton on top of the screen cover of the cage, or place a moist sponge or a small glass vial filled with water in the cage. The mouth of the vial is plugged with cotton and the vial laid on its side so the cotton remains moist. Pupae may be held for long periods in moist sawdust, vermiculite, sphagnum, or peat moss. In a flowerpot cage, the water used to keep the plant alive should provide sufficient moisture for the plant feeding insects and mites. Spraying the leaves daily also may supplement moisture requirements in rearing cages. Too much moisture may result in water condensation on the sides of the cage, which may trap the specimens and damage or kill them. Excess moisture also enhances the growth of mold and fungus, which is detrimental to the development of most insects and mites. A 2-3 percent solution of table salt sprayed regularly in the cage will help prevent mold and fungus growth.

1.13.2.2 - Temperature

Of all the environmental factors affect ing the development and behavior of insects and mites, temperature may be the most critical. Since arthropods are cold blooded, their body temperatures are usually close to the temperature of the surrounding environment, and their metabolism and development are directly affected by increases and decreases in temperature. Each stage of an insect or mite species has a low and a high point at which development ceases. These are called threshold temperature levels.

Most species that are collected and brought indoors for rearing can be held at normal room temperature; the optimum temperature for rearing will vary from species to species and with different stages of the same species. As with all rearing techniques, every attempt should be made to duplicate natural conditions. Specimens that normally would overwinter outdoors should be kept during the winter in rearing cages placed in an unheated room, porch, or garage. Never place an enclosed rearing cage in direct sunlight; the heat becomes too intense and may kill the specimens.

1.13.2.3 - Dormancy and Diapause

Insects and mites are unable to control the temperature of their environment; instead, they make physiological adjustments that allow them to survive temperature extremes. In regions with freezing winters, insects and mites have at least one stage that is resistant to low temperatures. The resistant form may be any stage—egg, larva, nymph, pupa, or adult. When winter arrives, only the resistant form survives. Dormancy is the physiological state of an insect or mite during a period of arrested development, whereas diapause is the prolonged period of arrested development brought about by such adverse conditions as heat, drought, or cold. This condition can be used to advantage in rearing. For example, if leaving rearing cages unattended for several days or longer is unavoidable, many (but unfortunately not all) specimens can be refrigerated temporarily to slow their activity and perhaps force diapause. This measure should be used with caution since the degree and duration of cold tolerated by different species will vary.

The reverse situation, that of causing diapause to end, is equally useful. Overwintering pupae that normally would not develop into adults until spring can be forced to terminate diapause early by chilling them for several weeks or months, then bringing them to room temperature so normal activity will resume. Often mantid egg cases are brought indoors accidentally with Christmas greenery. The eggs, already chilled for several months, hatch when kept at room temperature, often to the complete surprise and consternation of the unsuspecting homeowner.

1.13.2.4 - Light

Most species of insects and mites can be reared under ordinary lighting conditions; however, artificial manipulation of the light period will control diapause in many species. If the light requirements of the species being reared are known, it may be possible to adjust the period of light so that the specimens will continue to develop and will remain active instead of entering diapause, for example, providing 8-10 hours of light as opposed to 16 hours. Light and dark periods can be regulated with a 24-hour timing switch or clock timer. The timer is set to regulate light and dark periods to correspond with the desired lengths of light and darkness. It is important to remember that many insects and mites are very sensitive to light; sometimes even a slight disturbance of the photoperiod can disrupt their development.

1.13.2.5 - Food

The choice of food depends on the species being reared. Some species are general feeders and will accept a wide assortment of food, including dead or decaying organic matter. Examples of general feeders are most ants, crickets, and cockroaches. Other groups are specific feeders, with food preferences so restricted that only a single species of plant or animal is acceptable. Carefully note at the time of collection the food being consumed by the specimen, and provide the same food in the rearing cages.

Carnivorous insects should be given prey similar to that which they normally would consume. This diet can be supplemented when necessary with such insects as mosquito larvae, wax moth larvae, mealworms, maggots, or other insects that are easily reared in large numbers in captivity. If no live food is available, a carnivorous insect sometimes may be tempted to accept a piece of raw meat dangled from a thread. Once the insect has grasped the meat, the thread can be gently withdrawn. The size of the food offered depends on the size of the insect being fed. If the offering is too large, the feeder may be frightened away.

Bloodsucking species can be kept in captivity by allowing them to take blood from a rat, mouse, rabbit, or guinea pig. A human should be used as a blood source only if it is definitely known that the insect or mite being fed is free of diseases that may be transmitted to the human.

Stored-product insects and mites are easily kept alive and breeding in containers with flour, grains, tobacco, oatmeal or other cereal foods, and similar products. Unless leaf-feeding insects are kept in flowerpot cages where the host plant is growing, fresh leaves from the host plant must usually be placed in the rearing cage daily and old leaves removed.

1.13.2.6 - Artificial Diets

Some species can be maintained on an artificial diet. The development of suitable artificial diets is complex, involving several factors besides the mere nutritional value of the dietary ingredients. Because most species of insects and mites have very specific dietary requirements, information regarding artificial diets is found mainly in reports of studies on specific insects or mites.

1.13.3 - Special Problems and Precautions in Rearing.



Fig. 16. Lepidoptera temporaily stored in paper and glassine.

Problems may arise in any rearing program. Cannibalism, for instance, is a serious problem in rearing predaceous insects and necessitates rearing specimens in individual containers. Some species resort to cannibalism only if their cages become badly overcrowded. Disease is also a problem. It can be caused by introducing an unhealthy specimen into a colony, poor sanitary conditions, lack of food, or overcrowding.

Cages should be cleaned frequently and all dead or unhealthy specimens removed. Use care not to injure specimens when transferring them to fresh food or when cleaning the cages. Mites and small insects can be transferred with a camel's hair brush.

Attacks by parasites and predators also can be devastating to a rearing program. Carefully examine the host material when it is brought indoors and before it is placed in the rearing containers to lessen the possibility of predators and parasites being introduced accidentally. Also, place rearing cages where they will be safe from ants, mice, the family cat, and other predators.

References (rearing): Banks et al. 1981; Clarke 1941; Fincher & Stewart 1968; Furumizo 1975; Gerberich 1945; Harwood & Areekul 1957; Hodgson 1940; Krombein 1967; Levin 1957; Merritt et al. 1978; Peterson 1964; Sladeckova 1962; USDA 1970.

Part 2. - Specimen Preservation

2.1 - Liquid Agents for Killing and Preserving

Insects and mites of all kinds may be killed and preserved in liquid agents, but it is first necessary to determine the advisability of using a liquid killing agent rather than a dry gaseous agent. Some kinds of insects are best kept dry; it may not be advisable to allow others to become dry. Directions for the treatment of various insects are given in the last part of this publication under the various orders.

Ethanol (grain or ethyl alcohol) mixed with water (70 to 80 percent alcohol) is usually the best general killing and preserving agent. For some kinds of insects and mites, other preservatives or higher or lower concentrations of alcohol may be better. Because pure ethanol is often difficult to obtain, some collectors use isopropanol (isopropyl alcohol) with generally satisfactory results. Isopropanol does not seem to harden specimens as much as ethanol, and at least it is satisfactory in an emergency. Although there is controversy over the relative merits of ethanol and isopropanol, the choice of which to use is not so important as what concentration to use. This choice

depends on the kind of insect or mite to be preserved.

Parasitic Hymenoptera are best killed and preserved in 95 percent alcohol. This high concentration prevents the membranous wings from becoming twisted and folded, hairs from matting, and soft body parts from shriveling. This concentration may also be desirable if large numbers of insects are to be killed in a single container, such as in the killing jar of a Malaise trap, because the insect body fluids will dilute the alcohol. On the other hand, soft-bodied insects, such as aphids and thrips, small flies, and mites, become stiff and distorted if preserved in 95 percent alcohol and should be preserved in alcohol of a lower concentration. Adult bees should not be collected in alcohol because their usually abundant body hairs become badly matted. Adult moths, butterflies, mosquitoes, moth flies, and other groups with scales and long, fine hairs on the wings or body may be worthless if collected in alcohol regardless of the concentration.

Formalin (formaldehyde) solutions should not be used because the tissues become excessively hardened and the specimens then become difficult to handle.

Larvae of most insects should be collected in alcohol and subsequently killed in boiling water to “fix” their proteins and prevent them from turning black. Larvae should be left in hot water for 1-5 minutes, depending on the size of the specimens, then transferred to 70-80 percent alcohol. Large specimens or small specimens that have been crowded into one vial should be transferred to fresh alcohol within a day or two to reduce the danger of diluting the alcohol with body fluids. If the alcohol becomes too diluted, the specimens will begin to decompose. Water is not a preservative.

For some groups, preservation is better if certain substances are added to the alcohol solution. Thrips and most mites, for example, are best collected in an alcohol-glycerin-acetic acid (AGA) solution, and for many larvae a kerosene-acetic acid-dioxane (KAAD) solution is preferred. If KAAD is used, larvae need not be killed in boiling water. Formulas for these and other solutions are given in the Appendix.

For some histological, cytological, or physiological studies, specimens must be in a certain critical condition and must be preserved in special media (see Walker & Boreham 1976).

Larvae and most soft-bodied adult insects and mites can be kept almost indefinitely in liquid preservatives; however, for a permanent collection, mites, aphids, thrips, whiteflies, fleas, and lice usually are mounted on microscope slides (see p. 36). Larvae are usually kept permanently in alcohol, but some may be mounted by the freeze-drying technique (see p. 34) or by inflation (see p. 34).

Many insects collected in alcohol are later pinned for placement in a permanent collection. Hardbodied insects such as beetles can be pinned directly after removal from alcohol, but for them and all softer insects such as flies and wasps special procedures must be followed.

2.2 - Temporary Storage of Specimens

After specimens have been collected, time is often not immediately available to prepare them for permanent storage. There are several ways to keep them in good condition until they can be prepared properly. The method used depends largely on the length of time that the specimens may have to be stored temporarily.

2.2.1 - Refrigeration and Freezing

Medium to large specimens may be left in tightly closed bottles for several days in a refrigerator and still remain in good condition for pinning as will smaller specimens if left overnight. Some moisture must be present in the containers so that the specimens do not become “freeze-dried,” but if there is too much moisture, it will condense on the inside of the bottle as soon as it becomes chilled. Absorbent paper placed between the jar and the insects will keep them dry. When specimens are removed for further treatment, place them immediately on absorbent paper to prevent moisture from condensing on them.

Insects may be placed in alcohol, as described previously, and kept for several years before they are pinned or otherwise treated. However, it has been shown that many insects, especially small ones, can deteriorate in alcohol stored at room temperature. Long term storage of specimens that suffer from this kind of deterioration can be lessened by storing the containers in a freezer. Even though the alcohol will not freeze at the temperatures obtained by most ordinary freezers, the lower temperature seems to slow or stop deterioration of the specimens.



Fig. 17. Alcohol storage jars.

2.2.2 - Dry preservation

It is standard practice to place many kinds of insects in small boxes, paper tubes, triangles, or envelopes for an indefinite period, allowing them to become dry. It is not advisable to store soft—bodied insects by such methods because they become badly shriveled and very subject to breakage. Diptera should never be dried in this manner because the head, legs, and most of all the antennae become detached very easily.

Almost any kind of container may be used for dry storage; however, tightly closed, impervious containers of metal, glass, or plastic should be avoided because mold may develop on specimens if even a small amount of moisture is entrapped. Nothing can be done to restore a moldy specimen.

Dry-stored specimens must be labeled with complete collection data in or on each container. Avoid placing specimens collected at different times or places in the same container. If specimens with different collection data must be layered in the same container, include a separate data slip with each layer.

To insure that specimens do not slip from one layer to another, cut pieces of absorbent tissue, glazed cotton, or cellucotton a little larger than the inside of the container. Place a few layers of this material in the bottom of the container, then a few insects (do not crowd them), then more layering material, and so on until the container finally is filled. If much space is left, use a little plain cotton, enoush to keep the insects from moving about but not enough to produce pressure that will damage them. To prevent parts of the insects from getting caught in the loose fibers, use plain cotton only for the final layer. Insect parts are very difficult to extract from plain cotton without damage.

One method of keeping layered specimens soft and pliable for several months includes the use of chlorocresol in the bottom of the layered container and a damp piece of blotting paper in the top. The container must be impermeable and sealed while stored; plastic sandwich boxes make useful containers to use with this method. Add about a teaspoonful of chlorocresol crystals to the bottom, cover with a layer of absorbent tissue, follow with the layers of specimens, then a few layers of tissue, and finally a piece of dampened blotting paper as the top layer. The cover is then put in place and sealed with masking tape. It is best to keep boxes of layered specimens in a refrigerator.

Reference: Tindale 1962.

Some insects, such as small beetles, should be glued to points (see p. 29) directly from the layers for permanent preservation, but if they are to be pinned or otherwise treated, they must be relaxed as described on page 25.

2.2.3 - Papering

Although pinning specimens when they are fresh is preferable, the storage method known as papering has long been used successfully for larger specimens of Lepidoptera, Trichoptera, Neuroptera, Odonata, and some other groups. It is a traditional way of storing unmounted butterflies and is satisfactory for some moths, although moths too often will have their relatively soft bodies flattened, legs or palpi broken, and the vestiture of the body partly rubbed off. To save space in most large collections, file Odonata permanently in clear plastic envelopes instead of pinning them.

Papering consists of placing specimens with the wings folded together dorsally (upper sides together) in folded triangles (fig. 16) or in small rectangular envelopes of glassine paper, which are the translucent envelopes familiar to stamp collectors. Glassine envelopes have become almost universally used in recent years because of the obvious advantages of transparency and ready availability. In many collections, glassine has become partly superseded by plastic. However, many collectors still prefer folded triangles of a softer, more absorbent paper, such as ordinary newsprint, and believe they are superior for preserving specimens. Specimens can become greasy after a time, and the oil is absorbed by paper such as newsprint but not by glassine. Moreover, glassine and plastic are very smooth, and specimens may slide about inside the envelopes during shipping, losing antennae and other brittle parts. Although softer kinds of paper do not retain creases well when folded, this shortcoming may be circumvented by preparing the triangles of such material well before they are needed and pressing them with a weight for a week or so. Triangles are easy to prepare.

Some Lepidoptera are most easily papered if first placed in a relaxing box (see p. 24) for a day or two. The wings, often reversed in field-collected butterflies, may then be folded the proper way without difficulty. Do not pack specimens together tightly before they are dried or the bodies may be crushed. Do not store fresh specimens immediately in airtight containers or plastic envelopes or they will mold. Write collection data on the outside of the envelopes before inserting the insects.

2.2.4 - Liquid Preservation

Preservation of insects in alcohol is a complex subject and like many things, it varies somewhat from one

group to another. For example, spiders preserve well in ethanol, but tend to become to flaccid in isopropyl. The opposite is true for many myriapods. If one specializes in an insect group suited to preservation in one or another kind or concentration of alcohol, consult specialists in that group or experiment to find what yields the best results.

In general, ethanol and isopropanol mixed with water is the most widely used preservation fluids. Most commonly, a mixture of 75% alcohol to 25% water is used. The water should be distilled to ensure a neutral PH and the solution should be thoroughly mixed since alchols and water do not mix easily by themselves. Additives should be avoided.

Special care should be taken with labels placed in alcohol. Paper should be high quality rag or linen and acid-free. The ink should contain vegetable gum (such as India inks) as these seem to withstand the constant exposure to the alcohol the best. Typewritten labels and computer generated (laser printed) labels are generally unacceptable. The best system is still professionally printed labels.

Shell vials plugged by cotton or with polyethylene stoppers are recommended. Avoid stoppers made from cork, rubber, or neoprene as they tend to degrade and/or leach chemicals into the alcohol. Shell vials are preferred over necked vials as it is easier to remove the specimen and the chance of damage is reduced. Each vial should be individually labelled with complete collection data.

The shell vials are kept in wide mouthed, gasketed bail-top jars with straight sides (fig. 17). Avoid metal screw caps, bakelite lids, greased glass, and ground glass as they may rust, warp, crack, leak, or allow the alcohol to evaporate. Generally it is recommended that each jar contain between 10 and 40 vials. Avoid glass-glass contact by plaing a folded paper towel in the bottom of each jar. Keep vials upright within jars. Each jar should be filled with alcohol to just below the level of the gasket. If material is going to be stored for long periods of time, the jars should be checked periodically and the alcohol topped off. Labels may also be placed on the outside of the jars to indicate the enclosed contents.

Light is the chief enemy of alcohol preserved material, and as a result, jars should be stored inside cabinets. Vibration can also damage specimens and cause lids and caps to come loose so it is a good idea to place cabinets in a location where vibration is at a minimum.

Fire safety is always an important consideration when storing or working with alcohol collections. Concentrations of vapors can be very hazardous so care should be taken that work areas are properly ventilated and that there is no source of open flame nearby. In larger collections, special cabinets, exits, and other precautions may be necessary to meet the fire code.

References (J. Coddington, personal communication; Roth, 1952; Levi, 1966; Jocqué, 1983).

2.3- Preservation for Molecular Studies

Systematists are increasing using molecular methods to study insect relationships, make identifications, and determine species limits. Some of these techniques, such as study of cuticular hydrocarbons, can be used on dried insects, even those stored in museum collections. However, many others require that specimens be treated so that DNA or other molecules are preserved. In general, specimens for molecular work should be collected in 95% or absolute (100%) ethanol (ethyl alcohol). It is best if specimens are thoroughly dehyrated by changing the alcohol at least a couple of time before the specimens are stored for any length of time. It is also advisable to keep specimens cold (frozen if possible). For more detailed information on specimen preservation for molecular work see Hillis, et al. (1996).

Part 3. Mounting Specimens

Specimens are mounted so that they may be handled and examined with the greatest convenience and with the least possible damage. Well-mounted specimens enhance the value of a collection; their value for research may depend to a great extent on how well they are prepared. Standardized methods have evolved over about 2 centuries in response both to the aesthetic sense of collectors and to the need for high quality research material. Although the style and technique of mounting may vary from one



Fig. 18. Commonly used specimen mounting tools include a pinning block, forceps, pins, points, glue, and scisssors.

worker to another, the basic procedures outlined here are widely accepted. Methods of preparation are subject to improvement, but in the interest of uniformity it is best to follow currently accepted practices until the superiority of other methods has been proved.

The utility of a mounted specimen—that is, how well it is preserved, how safe it is from damage, and how much of the specimen can be examined conveniently— is generally of more importance than its beauty. Research considerations should take second priority only with specimens mounted specifically for nontechnical display purposes.

Preparation of specimens for a permanent collection is discussed here except specimens to be kept permanently in a liquid preservative or in papers or envelopes (see Temporary Storage of Specimens). Specimens to be prepared for a permanent collection may be fresh, that is, their body tissues not yet hardened or dried; or they may have been in temporary storage and must be specially treated before mounting. Dry specimens usually must be relaxed, and those preserved in liquid must be processed so that they will dry with minimal distortion or other damage.

Equipment typically needed to mount specimens includes forceps, a pinning block, pins, paper points, scissor, glue, and specimen holder (fig. 18).

3.1 - Preparing Dry Specimens for Mounting

Any dry insect that is to be pinned must be relaxed, that is, remoistened enough to soften so that it will not break when the pin is inserted or so that parts of the specimen may be rearranged or repositioned. Insects, especially Lepidoptera, that are to have their wings spread should be relaxed even if they have been killed for only a short time. The muscles of Lepidoptera, once the stiffening of rigor mortis sets in, which occurs in a matter of minutes, are strong enough so that adjustment of the wings is difficult, but treatment in a relaxing chamber usually will make this procedure much easier. Eight hours in a relaxing chamber should suffice, but larger specimens may require 24 hours or more. Simply leaving specimens in a cyanide jar for awhile sometimes will relax them, but this method is not reliable.

Reference: Lane 1965.

High humidity must be provided in a relaxing chamber for periods varying from several hours up to about 3 days, depending on the circumstances, without the specimens actually becoming wet. The growth of mold is also to be avoided, since it will ruin specimens left too long in relaxing chambers unless a chemical mold inhibitor has been added. Insects killed with cyanide usually can be relaxed easily, but some killing agents, especially chloroform, ether, and carbon tetrachloride, may harden muscles to such an extent that the specimens are brittle and seemingly impervious to the humidity of the relaxing chamber. In Korea, for example, butterflies are injected in the thoracic muscles with very hot water through a fine hypodermic needle before spreading. Occasionally, however, some specimens can not be relaxed satisfactorily by any method.

Many kinds of receptacles can be used as relaxing chambers, including glass dishes or jars with covers (low, widemouthed jars or casserole dishes are excellent), tobacco or biscuit tins, even earthenware crocks. Glass or earthenware containers are not so immediately affected by fluctuations in temperature as are other types and thus may relax insects more evenly. Containers 5-15 cm deep are

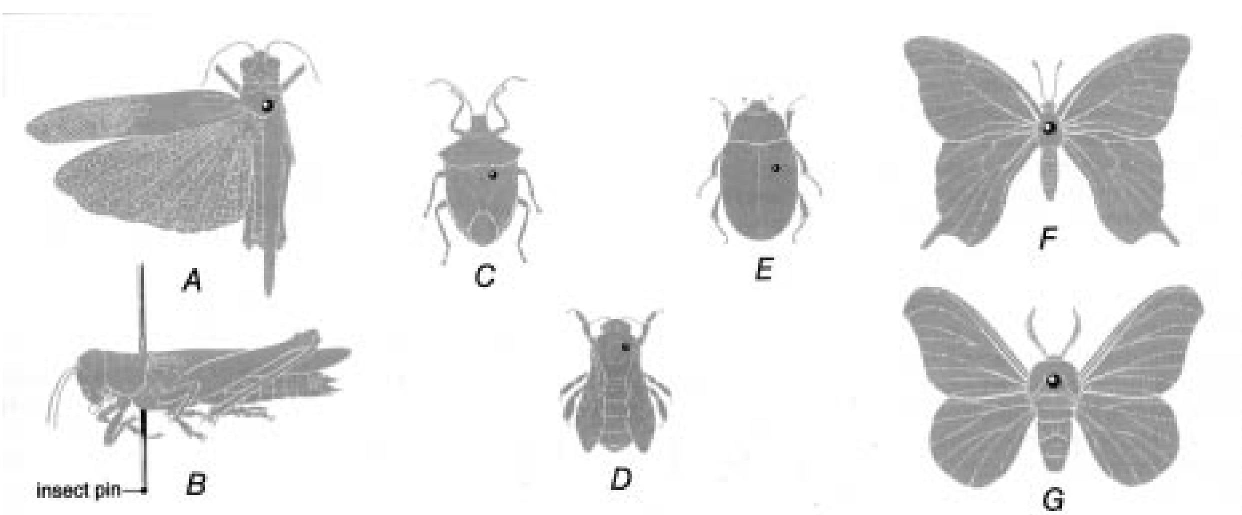


Fig. 19. Diagram showing the proper pin placement for mounting various types of insects.

most convenient; clear plastic sandwich boxes not more that 2.5 cm deep will serve for small specimens if they are not on pins. A layer of damp sand, peat, or crumpled paper toweling is placed in the bottom of the container and covered with a layer of cotton, cellulose wadding, or jeweler's cotton. This layer will not absorb water readily and will prevent direct contact between the insects and the moisture beneath.

Some workers object to the use of cotton because of the tendency for insect legs to become entangled in it and break off. If this is a problem, cover the cotton with a single piece of soft tissue. For very small specimens, a lining of tissue or some absorbent material with a smooth surface is advantageous. Heavy paper such as blotting paper or cardboard may be used in place of cotton, but this should be supported 1 cm or more above the moist bottom layer to avoid direct contact. Wooden or plastic strips or fine-mesh plastic screen also may be used for this purpose.

Mold probably will not be a problem if insects are relaxed for no longer than 2 days at normal room temperature, but relaxing chambers in regular use should be kept clean, with frequent renewal of the contents. If mold is likely to develop, as may happen with large specimens held more that 2 days, a few crystals of naphthalene, paradichlorobenzene, phenol, or chlorocresol may be sprinkled in the bottom of the relaxing chamber, or a little thymol, which is more potent, may be used. Al1 these chemicals, however, may damage plastic boxes.

Insects held too long in a killing jar, or those that were originally papered, pinned but unspread, or layered (that is, placed in small boxes between pieces of soft tissue) may be relaxed by placing them in a relaxing chamber. Papered specimens will relax faster if removed from their envelopes. For beetles and other insects that do not need to have the wings spread, holding them overnight or at most for 24 hours in a relaxing chamber will suffice. Small moths and delicate Neuroptera also should be relaxed sufficiently after 12-24 hours to allow the wings to be spread. Large moths, however, may take 48 hours or longer if the relaxing chamber is kept at room temperature. The process can be hastened and the chance of mold developing greatly reduced if the relaxing jar is subjected to a slight raising and lowering of temperature, as perhaps between 18° and 27° C. The process is greatly accelerated if the relaxing jar is set in, or floated on, warm water for an hour or more; specimens may be relaxed within 3-6 hours in this way. If the warm-water treatment is overdone, the specimens may be spoiled by the absorption of too much moisture. Some colors, especially nonmetallic greens in Lepidoptera, are unstable and may be completely bleached by exposure to too much humidity. Such material requires special attention; the specimens should be left in the relaxing chamber for the shortest possible time and ideally should be pinned and spread when fresh. Experience soon enables one to judge the best procedure for the particular kind of material being prepared.

The length of time that insects may be left safely in a relaxing chamber depends somewhat on the temperature. At 18°-24° C, they may be left for about 3 days, but beyond that time, they will begin to decompose. If the relaxing chamber is placed in a refrigerator at 3°-4°, the specimens may be kept for 2 weeks, although they may be slightly damaged from excessive condensation by that time. If relaxing chambers containing fresh specimens are placed in a deep freeze at -18° or lower, the specimens will remain in comparatively fresh condition for months, but not indefinitely. Specimens gradually desiccate and eventually will become dried. However, a freezer may be used to keep them fresh for a month or two and is a great convenience.

Even when specimens have been relaxed suitably for spreading, the wings may still seem stiff. In this instance, the wing muscles must be loosened by forcing the wings to move up and down. This may be safely done by pressing the tips of curved forceps firmly against the costal vein very near the base of the wing. The forceps should have the tips ground or honed smooth and not too sharp. Repeat this procedure separately with all four wings or they will revert gradually toward their original positions. With care, all the wings may be loosened in this way without leaving any visible marks.

Occasionally it may be desirable to relax and reposition only a part of an insect as, for example, moving a leg that may be concealing characters needed for identification. This may be accomplished by putting a drop or two of Barber's fluid (see Appendix) or ordinary household ammonia directly on the leg. Most household ammonia is now furnished with a detergent, which helps it wet and penetrate insect tissue. After a few moments,

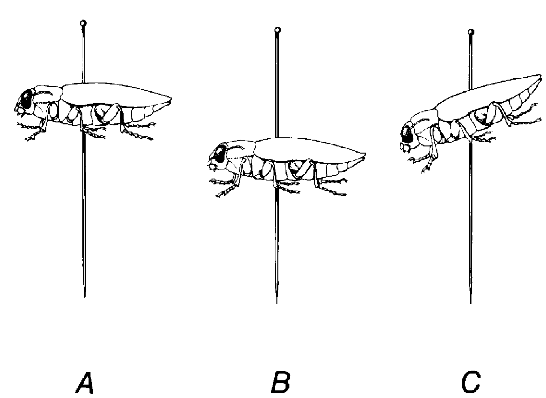


Fig. 20. Proper specimen placement on the pin. A) correct height and position. B) Specimen too low on pin. C) Specimen improperly tilted on pin.

perhaps after adding a little more fluid, the part may be pried carefully with a pin. When it moves easily, it may be placed in the desired position, held there with a pin fixed in the same substrate as is holding the pin on which the specimen is mounted, and left until the fluid dries thoroughly.

A few methods of relaxing insects with heat have been used. These and others are summarized in the following reference, in which a steam-bath method is described.

Reference: Weaver & White 1980.

3.2 - Preparing Liquid-Preserved Specimens

Most specimens preserved in fluid must be removed from the liquid in which they have been stored so that they will dry with as little distortion or matting of hairs as possible. Specimens which have been in fluid for some time should generally be washed with clean solution before drying. Only specimens with hard exoskeletons, such as beetles and some bugs (Pentatomidae, Cydnidae), may be mounted without special treatment when removed directly from the preserving fluid onto pins or points. The following methods have been used routinely for removing specimens from the usual fluid preservatives, and the specimens are often left in better condition than if they had been pinned while fresh, especially small Diptera.

The following equipment is needed: (1) A few screw-top jars about 5 cm in diameter with a cork cemented with epoxy on the top of each lid and a label on the outside showing clearly what they contain -- some about one-third full of Cellosolve (2-ethoxyethanol, ethylene glycol ethyl ether) and some about one-third full of xylene; (2) a small dish, such as a watchglass; (3) absorbent tissue from which to twist small "pencils" for absorbing xylene; (4) insect pins or double mounts (see p. 29)for mounting the specimens; (5) adhesive in a jar with a rod in its stopper; (6) narrow-pointed forceps; and (7) a few small cards of blotting paper.

When specimens are ready for preparation, remove as many from the preservative as can be pinned or placed on triangles or card points in an hour (experience will tell). Place them on blotting paper, then drop them from the blotter into a jar of Cellosolve, and place a label with the collection data on the pin stuck into the cork cemented to the lid of the jar. This may be done at the end of the day and the specimens left in the Cellosolve overnight, or otherwise for about 3 hours, longer for large specimens. They may even be left over a weekend. The same jar of Cellosolve may be used several times, up to about 10 times if the insects are small. This part of the treatment removes water and other substances from the specimens. However, Cellosolve does not evaporate readily, so it must be removed subsequently with another solvent, which will evaporate readily.

The next step is to use forceps to remove the specimens carefully from the Cellosolve, place them again briefly on blotting paper, then into a jar containing xylene. The identifying label on the pin in the cork must also be transferred. Small specimens should be left in the xylene for about 1 hour, larger specimens for up to 4 hours. Specimens left too long in xylene will become extremely brittle and can hardly be put on a pin or triangle without losing legs, antennae, or the head. As with the Cellosolve, the xylene also may be used many times until it becomes so contaminated with Cellosolve that specimens dry slowly when removed from it. While specimens are still wet with Cellosolve or xylene, they are somewhat pliable, and legs and antennae may be repositioned slightly.

When specimens have been in the xylene for at least 1 hour, they may be mounted. Take the smallest ones first to avoid leaving them in the xylene too long. Remove them with small forceps and place them in a dish. The forceps will pick up a small amount of xylene, and the specimen will be left lying in it. While there, it may be positioned correctly for mounting; the wings will float out flat, sometimes with a little adjustment with a pin or the tip of the forceps. When it is positioned correctly, take a "pencil" of absorbent tissue and touch it to the specimen to remove the excess xylene. Larger specimens may be pinned directly in the usual manner (see p. 28). Just before the xylene fully dries from the surface of a small specimen, the tip of a triangle or a tiny pin called a minuten, already attached to its carrying insect pin, should be touched to adhesive (see Double Mounts, p. 29). The tip of the triangle may then be touched to the specimen, picking it up. If a minuten is used, it may be inserted into the thorax of the specimen. A little final adjustment of position may then be made, and the specimen is ready for its label and place in the collection. If the specimen has been placed on a minuten, having touched the tip of the minuten to the adhesive will leave a small amount of adhesive around the place where the minuten has pierced the specimen and will keep it from working loose when fully dried.

Specimens placed on regular pins should have a small amount of adhesive placed around the site where the pin protrudes from the lower side of the specimen. Specimens pinned after having been in fluid preservatives do not cling as firmly to the pin as do those pinned fresh.

This treatment will leave surface pile, hairs, and bristles in a loose, unmatted, natural condition. Small specimens that shrivel considerably after having been pinned fresh will usually dry in better condition if pinned

or placed on triangles after this treatment.

Warning: Xylene is now considered to be carcinogenic. A new and already widely used chemical, Histo-Clear, is a promising substitute.

Reference: Sabrosky 1966.

3.3 - Direct Pinning

This section pertains entirely to insects because mites should never be mounted on pins. Direct pinning refers to the insertion of a standard insect pin directly through the body of an insect. Only insect pins should be used; ordinary straight pins are too short and thick and also have other disadvantages. Standard insect pins are 38 mm long and range in thickness from size 000 to 6 or 7. Heads are now commonly made of nylon, but they may be of a type called "upset," that is, an integral head is made by mechanically squeezing out the end of the pin, or a small piece of metal is pressed onto the pin. A well-made upset head is considered by some entomologists to be best; other kinds of heads sometimes come off, leaving a sharp point that easily can pierce a finger. Recently, however, pins have become available with nylon heads attached rather firmly. Pins of No. 2 diameter are most useful (0.46 mm in diameter). Most entomologists avoid the very slender pins of size 000 to 1, preferring to use double mounts (see p. 28), but now that soft polyethylene or plastic foam is commonly used for pinning bottoms in trays and boxes, these smaller sizes are not so impractical as formerly. Pins of larger diameter, Nos. 3-7, may be needed for large insects.

Standard insect pins are currently made of either ordinary spring steel, which is called 'black,' or stainless steel and with either a blued or a lacquered (japanned) finish. The black pins may corrode or rust with even slight exposure to moisture or to the body contents of the insects. Although the stainless steel pins are more expensive than black pins, their being rustproof makes them desirable for use in permanent collections. However, their points are somewhat more easily turned than those of black pins in piercing an insect with a hard cuticle, and they are not as rigid. For that reason, it is sometimes advisable to pierce an insect having an especially hard cuticle with a strong steel pin before inserting a stainless steel pin. Lacquered pins have a surface on which the insect may be less likely to become loose than it might on a bare pin.

Insect pins made of German silver or brass were once common. They quickly corroded from the action of the insect body contents, producing a greenish verdigris about the pin in the insect and eventually eating entirely through the pin.

One who handles a large number of pinned specimens may find pinning or dental forceps helpful. Their curved tips permit the pin to be grasped below the data labels and enable one to set the pin firmly into the pinningbottom material without bending the pin. The forceps are also of much assistance in removing pins tightly corroded into the cork pinning bottoms. The pin is grasped tightly above the cork and turned a little before it is lifted. However, with wings of most Lepidoptera, it is impractical to place pinning forceps below the specimen.

Insects should be pinned vertically through the body with a pin of appropriate thickness, using care that the pin does not tear off any legs as it goes through the body. Most insects are pinned to the right of the midline so that all the characters of at least one side will be visible. Figure 20 illustrates some right and wrong examples of pinning. Do not attempt to pin specimens unless they are relaxed (see p. 25) or freshly killed. Inserting a pin into a dry specimen may cause it to shatter. When pinning relaxed specimens or specimens taken from Cellosolve and xylene, a little glue may be . needed where the pin emerges from the specimen to prevent the specimen when dry from working loose and rotating on the pin. Application of adhesive is unnecessary when mounting freshly killed insects.

Standard methods of pinning some of the commoner types of insects are as follows:

(1) Orthoptera—Pin through back of thorax to right of midline (fig. 19, A—B). For display purposes, one pair of wings may be spread as shown, but many orthopterists prefer to leave wings folded because of limited space in most large collections (see Beatty & Beatty 1963).

(2) Large Heteroptera—Pin through triangular scutellum to right of midline (fig. 19, C). Do not spread wings. In Reduviidae, Coreidae, and other slender forms, pin through back of prothorax to right of midline.

(3) Large Hymenoptera and Diptera—Pin through thorax between or a little behind base of forewings and to right of midline (fig. 19, D). So that no characters on body are obscured, legs should be pushed down and away from thorax, and wings turned upward or sidewise from body. Wings of most Diptera will flip upward if specimen is laid on its back before pinning and pressure is applied simultaneously to base of each wing with pair of blunt forceps. Wings should be straightened if possible so venation is clearly visible. Folded or crumpled wings sometimes can be straightened by gentle brushing with a camel's hair brush dipped in 70 percent alcohol. For Hymenoptera wings, Peterson's XA mixture (xylene and ethanol, equal parts by volume) is recommended.

(4) Large Coleoptera—Pin through right wing cover near base such that the pin exits through the metathorax

(between the middle and hind legs) (fig. 19, E). Do not spread wings.

(5) Large Lepidoptera and Odonata—Pin through middle of thorax at thickest point (fig. 19, F) or just behind base of forewings (fig. 19, G). Spread wings as described on page 32.

The height of the insect on the pin will depend somewhat on its size, but enough of the pin should always be exposed above it to be grasped without the fingers touching and possibly damaging the specimen. Those mounted too high on a pin very likely will be damaged in handling. If pinned too low, the legs may be broken when the pin is inserted in a tray or box and insufficient space may be left for labels.

After the pin is inserted and before the specimen is dry, the legs, wings, and antennae should be arranged so that all parts are visible for study. With most insects, it is necessary only to arrange the legs and antennae in the desired position and let them dry, but occasionally it is necessary to hold the appendages in place with insect pins until the specimen is dry. With long-legged species or those with drooping abdomens, the legs and abdomens may be supported until dry with a piece of stiff paper pushed up on the pin from beneath. Once the specimens are dry, this paper support can be removed. For moths, butterflies, and other insects that should be mounted with the wings spread, use a spreading board (see p. 30) or spreading block (see p. 30).

Although some entomologists glue small insects directly to the side of a standard insect pin, this practice is not recommended because too much of the insect is often obscured either by the glue or by the pin, and the adhesive does not adhere well to the pin. For small insects, use a double mount.

3.4 - Double Mounts

Insects that are too small to be pinned directly on standard pins and yet should be preserved dry may be pinned as double mounts. This term refers to the insect's being mounted on a minuten or card point, which in turn is mounted or attached to a standard insect pin (fig. 21). Minutens are available from supply houses in 10 and 15-mm lengths and in two or three thicknesses. They are finely pointed at one end, headless on the other, and generally of stainless steel. Double mounts are assembled by inserting the minuten into a small cube of soft, pithy material such as fine cork, balsa wood, fine-textured plastic, or polyporus, which is a pure white material obtained from a bracket fungus. Polyporus traditionally has been a favorite material, but it is expensive and difficult to obtain, especially in America. Many entomologists prefer silicone rubber, obtained from plastics suppliers and made into plaques by pouring the polymerized material, a thick creamy liquid, into a flat- bottomed plastic container to a depth of about 2.5 mm and allowing it to solidify for several hours. It may then be lifted easily from the mold and cut with a sharp knife or razor blade into square strips and finally into cubes. With most materials, the minuten must be inserted point first, but with silicone rubber it may be inserted dull end first until it strikes the surface on which the cube is Iying, and it will be held firmly. Minutens should be handled with forceps; they are so small that even the unsharpened end can easily pierce a finger.

It is possible, and sometimes preferable, to mount an insect on a minuten before inserting the minuten into the mounting cube; however, it is most convenient to prepare a series of minuten mounts beforehand, already attached to standard No. 3 pins. To mount extremely small insects, such as tiny parasitic wasps, on minutens, pick up a droplet of cement with the prepared minuten and simply

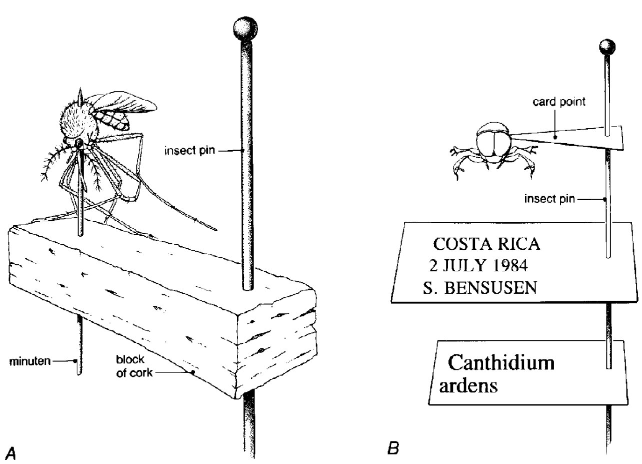


Fig. 21. Double mounts. A) A fly mounted on a minuten. B) A small beetle on a paper point.



Fig. 22. A card mounted chalcid wasp.

place the tip of the minuten with the cement on it between the base of the insect legs or on the right side of the thorax. In mounting an insect on a minuten, the pin need extend no more than barely through the insect. If the insect is lying on a glass surface when it is pierced with the minuten, a little extra pressure will curl the point of the minuten back into the insect and insure that the specimen will not come off the minuten.

For double mounting microlepidoptera, specimens are most easily mounted using a minuten, rather than a pin, and pinning and spreading the insects much as one would larger moths or butterflies. However, instead of using spreading boards (described later), a dense, smooth polyethelene foam is used as the spread surface. A narrow v-shaped groove cut into the foam provides a shallow indentation into which the body of the specimen can be placed so that the wings are level with the surface of the foam. Smooth polyethelene foam has a number of advantages for microlepidoptera. The surface acquires a small static charge which helps wings cling slightly to the surface and facilitates spreading; it grips the minutens firmly and leaves no holes; it sustains little wear over time. The foam is usually glued into clear, polystyrene plastic boxes. After the spread insects have dried, they are double mounted onto a polyporous block on a normal insect pin. Microlepidoptera should never be glues to points.

Many entomologists prefer to mount insects on a minuten in a vertical position in a short strip of polyporus or silicone, with the minuten therefore parallel to the main pin. The insect lies sidewise in the finished mount, in an excellent position for examination under a microscope, and is less liable to damage in handling than it would be otherwise.

Reference: Peterson et al. 1961; Landry and Landry 1994.

Card points are slender little triangles of stiff paper. They are pinned through the broad end with a No. 2 or 3 insect pin, and the insect is then glued to the point (fig. 21b). Card points may be cut with scissors from a strip of paper; they should be no more than 12 mm long and 3 mm wide. However, a special punch for card points, obtainable from entomological supply houses, will make better, more uniform points. Card points should be made only from good quality paper, as good as or better than that used for data labels (see p. 43). If specimens are in good condition and are well prepared, they may reasonably be kept in museum collections for a long time, perhaps even for centuries. Much of the paper in common use does not have that kind of life expectancy; it becomes yellow and brittle with age. Paper made especially to last, such as that used for herbarium sheets in botanical collections, is highly recommended.

A similar double mount method that is popular in Europe for mounting small specimens is the card mount (fig. 22). These small rectuangular cards can be purchased commercially or special punches can be bought to make them yourself. In this method the specimen is mounted laterally at about a 45 degree angle. If done properly, this technique allows viewing of all structures on the insect and the surrounding card provides greater protection for small and fragile specimens. However, caution must be used to ensure that the specimens's mouthparts, wings, etc. do not become embedded in the mounting media and that other characters are not obscured by mounting the specimen flat on the dorsum or venter. With some practice, this method provides very good results. A derivative of this method which also has the advantage of providing extra protection to the specimen is to use a standard point mount and then attach a card mount below, but close to, the pointed specimen.

The choice of the best adhesive for card points may be equally important, but unfortunately the aging properties of various glues are not known. Ordinary white (casein) glue, clear acetate cement, or fingernail polish is used commonly.

Another medium in use for many years is viscous polyvinyl acetate. It is obtainable in granular, bead, or pellet form. A small quantity is placed in a bottle with a glass rod in its stopper and covered with absolute ethanol. It will dissolve in a day or two into a thick solution. If it is



Fig. 23. A double mounted moth.



Fig. 24. A typical spreading board for Lepidoptera.

too thick, it will “string out,” and more ethanol should be added. If it is too thin, the bottle should be left open to allow some of the ethanol to evaporate. After a period of use, the solution will also normally become too thick, and then more ethanol must be added. Specimens adhere very well to a pin or a point with this solution, and they may be removed with 95 percent ethanol.

Many entomologists use shellac gel (see Martin 1977) which is now available commercially. It has the benefit of remaining tacky for some time and not forming a "skin" like some water based media. This allows additional time to get the insect positioned correctly. It is alcohol soluble.

Whatever adhesive is used, it should not be permitted to get so thick that it “strings.” Should this happen, add a little solvent to the adhesive until it attains the proper consistency. Nor should it be so thin that it flows over a specimen. Only a small amount of adhesive should be used to glue the specimen to the card point, since excessive glue may obscure certain sutures or sclerites necessary for identification, just as the card point may conceal certain ventral structures if allowed to extend beyond the midline of the insect.

For most insects, the card point is attached to the right side of the specimen (fig. 21b), with the left side and midventral area clear. For better adhesion with some insects, the tip of the card point may be bent downward at a slight angle to fit against the side of the specimen. Only a very small part of the point should be bent. With a little practice it will be easy to judge how much of the point to bend and at what angle to fit the particular insect being mounted. As an alternative to bending the tip, it can simply be snipped off with scissors to form a truncated end that fits each specimen, i.e., matching the size fo the metasternum in small beetles.

One method to insure that the specimen is oriented properly on the point is to place it on its back with its head toward you; then with the pin held upside down, touch a bit of adhesive to the bent point and apply it to the right side of the insect. If the top of the point can be slipped between the body of the insect and an adjacent leg, a stronger mount will result. The card point should be attached to the side of the thorax, not to the wing, abdomen, or head. Some insects, such as small flies and wasps, are mounted on unbent points. Those working with small flies prefer to attach the card point to the left side of the specimen with the legs facing the pin.

Opinions differ on when to use direct pinning and when to use a double mount, and perhaps this is best determined through experience. A general rule of thumb is that if you can mount a small insect on a size 1 or 0 pin without damaging the specimen, do not use a double mount. Insects too heavy to be held on the point by adhesive yet too small to be pinned with standard pins may be attached to card points by puncturing the right side of the insect at the place where the card point normally would be placed and inserting into this puncture the tip of an unbent card point with a little glue on it. For puncturing specimens, use a needle ground and polished to make a small, sharp scalpel. Some specimens, such as moths, should never be glued to points; other specimens should never be pinned with minutens. The following suggestions will serve as a guide:

(1) Small moths, caddisflies, and neuropteroids—Mount on minuten inserted through center of thorax with abdomen positioned toward insect pin (fig. 23). Mount must be sufficiently low so that head of pin can be grasped easily with fingers or pinning forceps. Do not glue small moths to points. Ideally, such specimens should be spread in the conventional manner despite their small size.

(2) Mosquitoes and small flies (freshly killed)—Pin

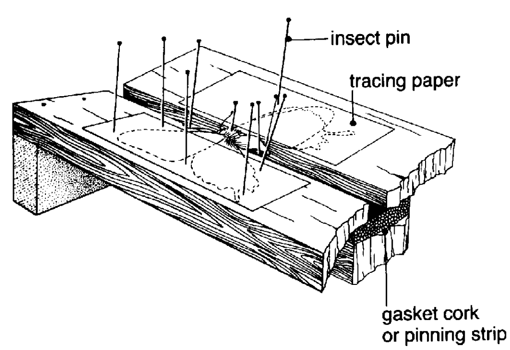


Fig. 25. Crosse section of a typical spreading board.

with minuten through the thorax with left side of specimen positioned toward main pin. Note that minuten is vertical, which is more advantageous than if it were horizontal because specimen is less liable to come into contact with fingers or pinning forceps. Placing a small amount of glue on tip of minuten before piercing specimen will help hold soft-bodied insects.

(3) Small wasps and flies (not freshly killed)—Mount on unbent card point with point inserted between coxae on right side of insect, keeping clear of midline, or glue tip of point to mesopleuron or laterally on a card (fig. 22).

(4) Small beetles, bugs, leafhoppers, and most other small insects—Glue card point with tip truncated or bent down to right side of specimen.

As to the length of pin exposed above the specimen, double mounts should conform to the same rule as in direct pinning: Do not place a double mount too high on the pin. It must be possible to grasp the head of the pin between the thumb and index finger without touching the specimen. Uniform height may be obtained by using a simple measuring device such as a three-step block prior to mounting the specimen (fig. 18). Double-mount cubes or points may be adjusted at any time, whereas once a directly pinned insect has dried on a pin, it is virtually impossible to move it without damage. If points become loose on the main pin, place a little adhesive at the connection.

Reference: Borgmeier 1964.

3.5 - Spreading Boards and Blocks

All insects preserved with the wings spread uniformly are set and dried in this position on spreading boards or blocks (fig. 24); spreading boards are more commonly used than spreading blocks. Although such pinning aids vary greatly in design, the same basic principle is inherent in all, that is, a smooth surface on which the wings are spread and positioned horizontally; a central, longitudinal groove for the body of the insect; and a layer of soft material into which the pin bearing the insect is inserted to hold the specimen at the proper height. An active collector will need from several to many spreading boards because the insects must dry for a considerable time (about 2 weeks for large specimens, one week for small ones) before being removed from the boards. Spreading boards may be purchased from biological supply houses or may easily be made as described here if the proper materials can be obtained. When purchasing spreading boards, avoid (1) too hard or too soft a material for the pinning medium under the central groove, (2) too hard an upper pinning surface, and (3) top pieces without the same thickness at the center (an especially common fault in beveled boards). This last defect may be corrected by sanding down the higher side; evenness is especially critical when working with small specimens.

3.5.1 - Construction of Spreading Boards.

A spreading board of simple design (fig. 25) requires the following materials:

(A) Two top pieces, 9 mm by 4.8 cm by 38 cm, preferably of seasoned basswood, a fine-grained, durable wood from trees of the genus *Tilia*. Holes made in it by insect pins tend to close after they are removed. If the surface of the board is lightly sanded after use, especially when working with small specimens, its smooth, even quality can be maintained through many years of use. Basswood is sometimes known as ‘whitewood’; however, wood from trees of the genus *Liriodendron* is also sold under this name. If basswood cannot be obtained, well-seasoned white pine selected for softness and ‘pinability’ is serviceable. A third choice is 20- cm (8-inch) beveled redwood siding. Beveled top pieces are desirable because the beveling, sloping inward, compensates for the tendency of the wings of spread specimens to droop slightly after the specimens are removed from the board. The 20-cm siding is actually 19.1 cm wide, and a 0.9- meter piece of it will provide two pairs of top pieces. One pair cut slightly more than 4.8 cm wide from the wide side of the board and planed to exact width will make a pair 11 mm thick at the narrow side, and another pair cut from the same side of the board will provide a second pair about 8 mm thick at the narrow side. Redwood is stiff and fine grained, but it splits and splinters easily.

(B) Two end pieces of any good, fine-grained wood, 2 cm square by 10 cm.

(C) One strip of entomological or gasket cork or foam, 6 mm by 3 cm by 34 cm.



Fig. 26. A small speading block.

(D) One base of plywood or any fine-grained wood, 6 mm by 1 0 cm by 38 cm.

These materials are for a spreading board with a central groove 6 mm wide. Boards with grooves of several sizes will be needed. For the larger Lepidoptera (macrolepidoptera or ‘macros’), the most useful widths are 3, 6, and 9 mm. For very large moths, a width of 17 mm is required; the board will also have to be as much as 15 cm in total width with a groove depth of 16 mm. For small moths (microlepidoptera or’microsi), special boards with groove widths of 1.5-2 mm will be needed, with the groove shallow enough for minutens, and the width and thickness of the top pieces (A) must be altered accordingly. The pinning medium (C) could be of polyethylene foam, but to give specimens firm support, the entire depth between the top pieces and the base would have to be filled with the material. A dense, finely textured plastic foam known as ‘Plastazote’ is better than polyethylene for entomological applications and is available in Britain but so far not in the United States. A strip of modelmaker’s balsa wood, selected for pinning softness, may also be satisfactory.

The end pieces (B) should be glued with epoxy or other good adhesive and nailed to the top pieces (A) with the proper groove width maintained. Then the pinning strip (C) should be firmly glued to the underside of the top pieces (A), the same side on which the end pieces (B) were fastened, and should cover the central groove. Finally, after the adhesive has set, the base (D) should be attached. If it is affixed to the end pieces (B) with two flat-headed wood screws (about No. 5,19 mm) countersunk into the base piece and screwed into each end piece, the base may be removed easily later if replacement of the pinning strip is necessary.



Fig. 27. A small speading board of the type used for microlepidoptera.

3.5.2 - Using the Spreading Boards

Before spreading specimens, the spreading boards and the following materials should be at hand:

(1) Pins (called setting pins) of size 00 or 000 for bringing wings into position. Setting pins used by some lepidopterists are made by inserting a minuten into a round matchstick and securing it with a drop of glue.

(2) Strips of glassine or tracing paper (the translucent, smooth paper used for tracing, not what a draftsman calls tracing paper). Cellophane, plastic film, or waxed paper should not be used. Their disadvantages include expanding with moisture and becoming electrostatically charged or containing a substance that pulls scales off the wings. The strips of tracing paper should be wide enough to extend from the base to a little beyond the end of the wings of the specimens being spread. Strips about 25 mm wide are convenient for spreading most Lepidoptera. Short ones are used when spreading specimens that have been relaxed from a dried condition, but strips long enough to cover several specimens in a row on the board are commonly used for freshly caught insects. The strips are often used with a narrow fold alongside the body of the specimen with the fold upward; this provides a rounded edge that reduces the likelihood of a sharp edge displacing a row of scales. This fold may be made by holding the strip on a spreading board with 3-5 mm of it overhanging the edge of board, running a finger along the overhang to bend it down, and then firmly folding it back.

(3) Glass-headed pins at least 2.5 cm long for holding the strips in place. Ordinary No. 2 or 3 insect pins with nylon heads may also be used, but some lepidopterists find them hard on the fingers.

With this equipment ready, the collector is prepared to mount and spread the specimens (fig. 20, B). The specimens must be properly relaxed (see p. 24), even the freshly collected ones, before any attempt is made to spread the wings. Insert an insect pin of appropriate size through the middle of the thorax, leaving at least 7 mm of pin above the specimen. The pin should pass through the body as nearly vertically as possible to avoid having the wings higher on one side than on the other. Pin the specimen into the central groove of the spreading board so that the wings are exactly level with the surface of the board. Carefully draw each wing forward with the point of a setting pin inserted near the base and immediately behind the strong veins that lie near the front of the wings. If care is taken not to tear the wing, the fine setting pins should leave holes so small that they are barely visible. The

hindmargin of the forewings should be at right angles to the groove in the board. Bring the hindwing into a natural position with its base slightly under the forewing. The setting pins will hold the wings in position until they can be secured with the paper strips.

The strip is placed close to the body of the insect, with its fold upward and toward the insect. A glass-headed pin is inserted in the middle of the folded part of the strip just outside the margin of the forewing. The pin may be tilted slightly away from the wing to keep the strip down against the wing. The strip is then carefully stretched backward and another pin placed just behind the hindwing. A third pin in the notch where the forewings and hindwings meet is usually enough. None of the three pins on each side of the specimen should pass through the wings. Once the paper strips are in place, the setting pins may be removed. Twisting the setting pins a little as they are removed will prevent a possible bent tip from hooking onto a wing vein and pulling the whole wing out of place.

Fresh specimens may be arranged closely on the board in series of five, six, or even more before the paper strips are applied to cover all. Relaxed specimens, however, should be treated individually because they dry so quickly that antennae may break or the wings curl if the spreading is not completed promptly.

To prevent the abdomen from drooping as the specimen dries, support it with a pin on each side, crossing beneath. Pins may also be used to arrange and hold the antennae and legs in position until they dry. The appearance of many insects may be improved by gently blowing on them before spreading to remove extraneous loose scales and to straighten the hairs or, with small moths, the fringes of the wings. In working with small insects, a large magnifying lens mounted on an adjustable stand may be very helpful.

Specimens relaxed from a dried condition present some additional problems. The wings may be stiff and require loosening (see p. 25). If the wings of a relaxed specimen are turned upward and do not lie on the surface of the spreading board, the paper strips may need to be pinned over tne wings to hold them down before they are positioned. Since the wings of relaxed specimens are still relatively stiff, skillful manipulation is needed to spread the wings without tearing or leaving excessively large pinholes. If the wings do not move readily under gentle pressure, do not force and possibly break them. Return the specimen to the relaxing chamber.

3.5.3 - Construction of Spreading Blocks.

The spreading block is a modification of the spreading board designed to accommodate only one specimen at a time. In the past, blocks were often preferred by specialists in microlepidoptera. More recently, most specialists have taken tosing smaller spreading boards such as that in fig. 27. However, spreading blocks can used for other insects as well. The design is simple (fig. 26), consisting of a wooden cube about 3 cm on a side for most insects, with a groove across the middle of one face. The width and depth of the groove vary to suit the size of the insect to be spread, usually 1.5-2 mm in width and deep enough to accommodate a strip of fine cork, polyporus, or similar pithy material into which the pin or minuten is lodged to hold the insects being spread. The groove should be cut parallel with the grain of the wood, and the top surface of the block should be sanded exceedingly smooth. Before the pinning strip is wedged or cemented into the bottom of the groove, a hole about 1 mm in diameter should be drilled squarely in its middle. The pin can extend into this hole when the insect becomes level with the spreading surface. A few gashes, made by pressing the blade of a thin knife in the upper corners of the block near each end of the groove, should be made to catch the thread that will hold the wings of the insect.

The insect to be spread, mounted either on a standard insect pin or on a minuten, is pinned into the groove as with a spreading board, and the wings are manipulated by gentle blowing and using a setting pin. A piece of fine silk or nylon thread is then caught in one of the knife gashes and brought over the wings, and, if necessary, once around the block and again over the wing at another point, and finally caught again in the knife gash. A small piece of tracing paper may be placed on the wings before passing the thread over them, but if special scale tufts are found on the wings it is better to omit the paper and leave the tufts in a natural position.

Specimens either on spreading boards or blocks should be placed in a warm, well-ventilated place to dry for at about 2 weeks less for very small moths. If they are placed in a low- temperature oven, such as is used for drying plant specimens, 2 days may suffice. Specimens relaxed from a dry condition, as already noted, dry quickest, but even they should be left for several days. Fresh specimens, even large ones, may be dried in 2 days or less with heat. Where humidity is low and there is ample sunshine, the spreading boards or blocks may be placed in cardboard cartons painted black and left out in full sunlight for about 2 days. Occasionally, specimens may become greasy, but otherwise no harm results. The spreading boards or blocks must be kept where they are safe from mice, bats, dermestid beetles, lizards, psocids (booklice and barklice), and ants, especially in the Tropics. One preventative measure that is sometimes advisable is to place the boards or blocks on bricks set in pans of water. If they are hung from the ceiling, a mosquito net around them may be necessary.

Always keep temporary data labels with specimens on spreading boards or associated with them in some way to insure that there is no confusion or loss of data when they are removed from the boards.

Spreading is a highly individualistic skill, subject to wide variation. Nearly everyone, with practice, evolves his or her own technique, so that two workers may appear to follow different procedures and yet produce equally good results. There is no single standardized technique with respect to the fine points of spreading.

References: Lewis 1965; Tagestad 1974, 1977.

3.6 - Riker Mounts

It is sometimes desirable to prepare specimens for exhibition in such a way that they may be handled freely for close examination without risk of damage. Riker mounts have long been used for this purpose. They may be purchased from entomological supply houses, but similar cases may easily be constructed. The Riker mount (fig. 28) is simply a flat cardboard box about 3 cm deep, filled with cotton, and having a pane of glass or plastic set into the cover. Unpinned specimens are placed upsidedown on the glass of the cover, spread into position with some cotton held in place by small weights, and allowed to dry thoroughly (about 2 weeks). Then the weights are removed, enough cotton is added to hold the specimens firmly in place, a little fumigant is added to kill any pests or their eggs that might have been laid in the box, and the bottom part of the box is put in place. When the box is closed, it should be sealed completely to prevent access to pests. Plant material may also be dried in place with the specimens.

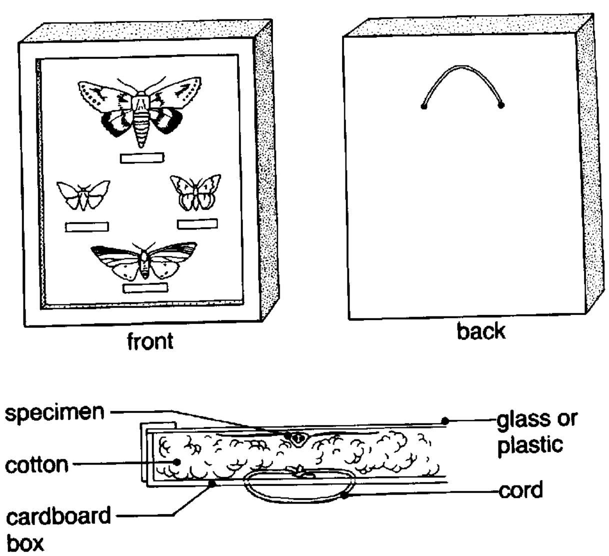


Fig. 28. A Riker mount.

Riker mounts are practical only for relatively large insects, such as butterflies, larger moths, beetles, and dragonflies, that are suitable for such display. Although Riker-mounted specimens are useful for classroom instruction and general display, they are not used for storage of insects in a scientific collection, where specimens must be available for examination from all angles under magnification. Riker mounts should be inspected periodically for pests and kept out of sunlight, which will cause fading of colors and general deterioration.

It is sometimes desirable to put pinned specimens into Riker mounts. To do so, remove the pin or cut it off flush with the surface of the insect (see Holbrook 1927).

3.7 - Inflation of Larvae

A common practice in the 19th and early 20th centuries was to preserve larvae, mainly caterpillars, by inflation. That practice has largely been abandoned in favor of alcoholic preservation or freeze-drying. These latter methods permit more thorough examination of all parts of the specimens, even internal organs, which must be removed before inflation. Some of the colors of larger larvae are better preserved in inflated specimens than in alcohol, but color photography has made preservation of the larval colors less essential. However, the technique is still potentially useful and, if well done, is not to be discouraged. For instructions on how to inflate larvae, the following references may be consulted.

References: Banks 1909 (pp.69-70); Hammond 1960; Martin 1977.

3.8 - Artificial Drying

The most widely used method of artificial drying



Fig. 29. A critical point dryer and its accompanying CO_2 supply.

now in use at most museums and other institutions is critical point drying (fig. 29). In critical point drying (CPD), specimens are immersed in absolute ethanol and a special machine is used to exchange the alcohol with liquid carbon dioxide under pressure. The liquid CO_2 is then warmed and passes through the "critical point" and is bled off. The effect of this process is to remove all water from soft tissues and in effect "freeze" them in position. In this way, soft bodied specimens can be dried without the distortion that normally results when soft tissues are air dryed. CPD machines are still fairly expensive and generally beyond the range of individuals although they are very common at larger institutions.

A more "low-tech" method of drying soft-bodied insects and other arthropods in a very lifelike manner and with no loss of color is by freeze-drying. While the cost of specialized freeze-drying equipment is high, it is possible to freeze dry specimens in an ordinary freezer if done carefully.

Briefly, the procedure consists of killing the insect by first freezing it in a natural position and then dehydrating it under vacuum in a desiccator jar kept inside a freezer at -4° to -7° C. With a vacuum of 0.1 micrometer at -7°, a medium sized caterpillar will lose about 90 percent of its moisture and about 75 percent of its weight in 48 hours. Its frozen condition prevents distortion while drying. The time required to complete drying is variable, at least a few days with small specimens and more than a week with larger ones. When dry, they can be brought up to room temperature and pressure, and permanently stored in a collection. Like all well-dried insect specimens, they are rather brittle and must be handled carefully. Freeze-drying yields excellent specimens of plant galls formed by insects.

An inexpensive method of freeze-drying (Fisher & Jursic 1958) requires about 100 days to dry a medium-sized larva. The use of acetone is recommended before drying pinned specimens for better preservation of their colors, one of the features sought in artificial drying (Berte 1979).

Another method of drying involves the use of hexamethyldisilazane (HMDS) (Brown 1993; Nation 1983; van Noort 1995). Using this method specimens are soaked in absolute alcohol until all water has been removed. They are then moved into a small amount of HMDS for a few minutes, then into a second bath of HMDS (larger specimens may require a third transfer), which replaces the alcohol in the specimen with HMDS. Finally, the HMDS is allowed to evaporate. This method has proven quite effective in preventing distortion of specimens, but HMDS can be toxic and must be used with adequate ventilation, preferably within a fume hood. A variation of this method uses acetone in place of HMDS.

References: Berte 1979; Dodge & Seago 1954; Fisher & Jursic 1958; Gordh & Hall 1979; Harris 1964; Hower 1979; Woodring & Blum 1963.

3.9 - Embedding

Preservation of various kinds of biological specimens in polymerized transparent plastics was popular in the 1940’s and 1950’s and is still of some interest. The process is rather complicated and laborious, but if carefully done it will yield useful preparations, especially for exhibits and teaching. Directions for embedding insect specimens may be found in the references belowand in directions furnished by suppliers of the materials.

References: Fallis & Smith 1964; Fleming et al. 1940; Hocking 1953.

3.10 - Mounting Specimens for Microscopic Examination

The small size of mites, thrips, whiteflies, aphids, scale insects, fleas, parasitic wasps, and many other insects, as well as the necessity of clearly seeing minute details of larger insects, requires examination under a compound microscope at high magnification. Such specimens and parts of specimens must therefore be specially prepared and placed temporarily or permanently on microscope slides. If large and thick or complex structures that must be examined from several angles make slide mounting inadvisable, they may be examined in a liquid and preserved in microvials. Whichever course is adopted, their preliminary treatment is the same.

The techniques and materials (fig. 30) used in preparing specimens for high-power microscopic examination vary considerably according to the kind of insect or mite as well as the researcher’s preferences. The information given here will provide the reader with a basic



Fig. 30. Typical materials used in slide making.

concept of the principles involved in preparing specimens for such study. For more specific instructions, consult the references cited below. For reagents and media formulas mentioned here, see the Appendix.

References (general): Baker 1958; Burrells 1978; Fredeen 1961; Gruber & Prieto 1976; Guyer 1953; Hood 1947; McClung 1964; Mitchell & Cook 1952; Noyes 1982; Richards 1964; Singer 1967; Willey 1971; Wirth & Martston 1968.

Although information on slide preparation is broad and varies considerably according to the condition of the specimen and the mounting medium used, certain features are common to all processes. Cleaning, clearing, and maceration are nearly always necessary preliminaries. It is often desirable to dissect and critically examine specimens after the preliminary treatment and before mounting.

Clearing is the process of making the tissues of the specimen more transparent. It is often advisable to remove internal organs and muscles by using chemicals and to extend, manipulate, or dissect the specimens. This chemical removal of muscles and other soft tissues is known as maceration, although it is sometimes incorrectly called clearing. The agents used to macerate specimens usually also clean and clear them. Many mounting mediums also act as clearing agents to some extent.

Reference: Hazeltine 1962.

Dehydration is usually a necessary preliminary to mounting, especially if the medium has a resin base. With some kinds of specimens, it is advisable to do this gradually or in steps to avoid distorting the specimens.

Staining is sometimes necessary with insect and mite specimens because their immersion in the mounting medium may make colorless and transparent tissues virtually invisible if the medium has a refractive index close to that of the tissues of the specimen (see Stein et al. 1968). Bleaching, usually accomplished with hydrogen peroxide, may also be required in very dark-colored specimens.

Washing is usually necessary at one or more stages in the process to remove and prevent excessive action by certain reagents used.

The final stage in preparing permanent mounts is thorough drying or hardening of the medium. This may be done in any clean environment or in an oven or special slide warmer under gentle heat. The mounts should be carefully labeled either before drying or afterward. If more than one mount is being made at a time, some recognition mark or code must be used on reagent containers and anything associated with the specimen so that the final mount may be correctly labeled.

The following procedures are given for mounting specimens to be examined microscopically:

(1) Maceration. Since only the sclerotic or chitinized parts of the insects are ordinarily needed in a preparation, the aim of maceration is to eliminate external secretions, foreign matter, some organs, muscles, and fat bodies without damage to chitinous parts. This is accomplished by immersing the specimen in a suitable agent, such as a sodium hydroxide (NaOH) solution, lactic acid, or lactophenol. These chemicals are strongly caustic and must be handled carefully to avoid damage to the skin and eyes. If any is inadvertently spattered on the skin, immediately wash it off with water.

Although textbooks specify potassium hydroxide (KOH) for maceration, this chemical must be used cautiously because specimens may be easily and quickly damaged or completely ruined in it. NaOH will perform as well as KOH or even better. Fine ducts lost with KOH remain even after lengthy treatment with NaOH, which will damage only teneral or newly emerged specimens. The amount of time a specimen is left in the macerating agent depends on the degree of sclerotization and the age and pigmentation of the specimen. For some relatively large, whole insects, the cuticle must be punctured with a fine needle to allow the agent to penetrate the body. Heating accelerates the action, but care must be taken to avoid damage by excessive action, especially if the specimen is at all teneral. Immersion of the genitalia in cold 10~20 percent KOH solution overnight is the recommended method for microlepidoptera. Boiling for a minute in a 10 percent solution of NaOH (ordinary household lye) will clear most other small genitalia. NaOH supplied by chemical firms in pellet form is most convenient; three



Fig. 31. Slide mounted specimens. The bottom specimen is mounted in Hoyer's medium and has been ringed to prevent dessication.

pellets in about 10 ml of water may be used for a day. The solution, however, is useless if left overnight. Even if it boils dry on a hotplate set a little above its boiling point, a specimen in it will seldom be damaged by NaOH, although it will be completely dissolved in KOH. Adding water to a specimen boiled dry in NaOH solution will usually restore it.

For directions on how to macerate insect genitalia, see p. 37.

(2) Washing. For the removal of the caustic agent used to macerate the specimen, ordinary tapwater in a small dish, such as a small plastic bottle cover, will suffice. Distilled water is unnecessary. If the specimen is placed for at least a few minutes in plain water for manipulation, dissection, or examination, it then will be ready for further treatment. Adding a drop of acetic acid (white vinegar) will guarantee that no caustic remains.

(3a) Staining. After clearing and washing, specimens may be stained if necessary, although if a phase-contrast microscope is available, staining, even with colorless specimens, is unnecessary. Several kinds of stains are available from biological supply houses. Acid fuchsin is generally used for aphids, lice, and scale insects. Chlorozol black or mercurochrome generally are used for microlepidoptera, although the latter may fade over time. Thrips and fleas should never be stained; most acarologists do not stain mites if they are to be mounted in Hoyer's medium. An easily obtained stain for the exoskeleton of insects is made by dissolving a small amount of Mercurochrome crystals in water. Specimens may be immersed in the stain solution for 1 minute or more, depending on the degree of staining needed, and then briefly rinsed in plain water.

References: Carayon 1969; Gier 1949.

(3b) Bleaching. If specimens are too dark to reveal sufficient detail after maceration, they may be bleached in a mixture of one part strong ammonia solution to six parts hydrogen peroxide solution. The length of time the specimen is left in the ammonia-peroxide solution depends on the amount of bleaching needed.

(3c) Dehyrdation. Specimens should be dehyrated (have the water removed) in alcohol or cellosolve. The length of time depends on the specimens, but 10-20 minutes is usually sufficient.

(4) Mounting. At this point, further treatment depends on what use is to be made of the preparation. It may be needed only temporarily in routine work and may be discarded after examination, or it may be desirable to keep the preparation permanently, either in glycerin in a microvial or in a mounting medium on a slide. If it is to be kept in a microvial, see Preparation and Storage of Genitalia (p. 37); if it is to be mounted on a slide, further treatment depends on the mounting medium used.

(4a) Temporary Mounting. A temporary mount can be made with lactic acid or other medium on a 2.5- by 7.5-cm cavity slide. The specimen is placed near the edge of the cavity and wedged into position by manipulating a cover glass over the cavity and the specimen. A fine needle will help bring the specimen into the desired position before the cover glass is centered over the cavity. Once the specimen is in position and the cover glass centered, a commercial ringing compound, nail polish, or quick-drying cement is used to seal around the edge of the cover glass. Such slides may be kept for a year or more, but because they take up more space in a collection than permanent slides, the specimens eventually are usually placed in vials of alcohol for storage.

Temporary mounts are advantageous in that the specimen can be turned easily and viewed from many angles. However, because of the thickness of the mounts, a vertical illuminator operated through a microscope or some alternate method of direct lighting is generally needed.

Genitalia and other insect or mite parts may be examined and drawings made with the aid of an ocular grid in the microscope while they are lying in water in the dish in which they were dissected and extended. Water gives contrast to the structure, which may be difficult to see in glycerin. The water should be "dead," that is, boiled to drive out gases that may form bubbles in or on the object. The object may be held in place with a minuten bent L-wise and laid over the object or by piercing it at a convenient place.

(4b) Mounting Media. The standard medium for permanent mounts is Canada balsam. Before mounting, the specimen must first be dehydrated through a series of alcohols of increasing concentration or in cellosolve. Balsam may yellow somewhat with age and this can make observation of characters and photography difficult; it can also be difficult to manipulate delicate specimens in it if it is not thinned properly. The mounting medium should be selected after consulting with a specialist or by referring to textbooks. Mites, for example, require special treatment, mainly because their cuticle differs from that of insects.

Another satisfactory mounting medium for most insects (other than scales and thrips) is Euparal, a synthetic preparation used for many decades. When it was unobtainable, especially during the World Wars, an inferior compound was used. Euparal may be obtained from medical or entomological supply houses and other sources, all of which import it from Germany. Its formula is a proprietary secret. It is not necessary to dehydrate specimens before mounting them in it. Good preparations may

be made from specimens taken directly from 80 percent ethanol and from specimens immersed in 95 percent ethanol for only a minute.

The medium is water-white, remains so indefinitely, and for remounting, in case of breakage, specimens may be removed by soaking in absolute ethanol. Euparal has a very decided advantage over other media in that small air bubbles trapped in slide preparations are absorbed by the medium during drying, although this sometimes requires several days. Its only disadvantage is that it shrinks considerably in drying. In moderately thick preparations, this results in shrinkage away from the edges of the cover slip. This may be countered by adding additional Euparal until there is no further shrinkage, or in many instances by using a large cover slip, 2.2 cm in diameter, which in drying will pull down around the edges instead of allowing the medium to draw inward. The medium is relatively fast-drying. Allowing the slide to remain overnight in an oven set at about 35° C or in the open at room temperature for a few days will yield usable and permanent preparations.

Hoyer's medium and polyvinyl alcohol (PVA) are aqueous mounting media. Slides made with them are considered only semipermanent, although in the U.S. National Collection of Insects at the Smithsonian Institution, some 40-year-old slides of mites mounted in Hoyer's medium are still in good condition. Nevertheless, many other slides show significant deterioration after only a few years, even when ringed. Slide preparations made with Hoyer's or PVA, particularly of large or thick specimens, tend to crystallize with age and may need remounting. Some specimens may be destroyed completely.

To remount specimens, soak the slide in water until the cover glass can be removed, then lift the specimen carefully and transfer it to a new slide. Some technicians find slides easier to prepare if the Hoyer's medium is diluted with water; however, in the process the mounts may collapse as the excess water evaporates. It is strongly recommended that Hoyer's medium be prepared exactly as directed (see Appendix) and used undiluted. However, it should be noted that one of the primary ingredients (chloral hydrate) of Hoyer's is now listed as a controlled substance by the government so that it is impossible to buy without a permit.

Aqueous media are affected by ambient moisture; mounts made in very humid conditions may not dry satisfactorily. Nevertheless, Hoyer's is preferred by most acarologists because its refractive index is excellent for use with mites, and specimens can be mounted directly from the collecting fluid without clearing or fixing. The specimens are cleared after mounting by heating the slides briefly on a hotplate set at 65° C until the medium barely begins to bubble. Do not allow Hoyer's medium to boil or the specimens may be ruined. Such mounts can be prepared quickly for immediate study but should be placed in an oven for curing (See item (7).

(5) To place specimens in the medium, put one or more drops of the medium in the center of a 2.5- by 7.5-cm clean glass slide. The precise amount of medium to use will require some experience. Enough is needed to run under the entire cover glass. When Euparal is used, a little more is required than with some other media because of shrinkage, but an excess of any medium around the edge of the cover slip is undesirable.

Place the cleared and washed (also stained or bleached if necessary) specimen in the medium on the slide and make sure that it is well immersed and that air bubbles are absent. Arrange it in the desired position with a fine needle. If the specimen is thick, place at least three pieces of broken cover glass or plastic props around it to prevent undue crushing when the cover slip is applied. With some preparations, as for example with ovipositors of tephritid flies, a considerable amount of pressure during drying is desirable to obtain maximally flattened and comparable preparations. Then gently lower a cover slip onto the specimen with forceps, holding the cover slip at a slight angle so that it touches the medium first at one side to prevent air entrapment as much as possible. A small amount of thinning agent on the under surface of the cover slip may help avoud trapped air bubbles. Apply gentle pressure with the forceps to fix the position of the specimen.

It is often advisable to prepare specimens in more than one position, for example, dorsal side up as well as dorsal side down, but do not mount parts of more than one individual specimen on one slide, because all individuals in the series may not be taxonomically identical.

(6) Ringing. Special compounds are available to apply in a circle around the edge of the cover glass and the adjacent area of the slide to seal the medium (fig. 31). This is advisable with aqueous and other median that do not harden as they dry. It is not necessary to ring Canada balsam or Euparal mounts.

(7) Curing. Allow slides to dry or set completely before handling or placing them in other than a horizontal position. Until dry, avoid storing them in a slide box, mailing them, or allowing other persons to use them. If an oven or slide warmer is available, set it at about 45° C. The amount of time it takes to dry a slide is variable depending on the medium, size of the specimen, and other factors such as humidity. It may take from a couple days for small specimens in aqueous media up to several weeks for larger balsam mounts before a slide is dry enough to ship or store on edge. One way to check progess is by very lightly pressing on the center of the coverslip and watching

to see if there is movement of the specimen in the medium.

(8) Labeling Slides. Collection data should accompany specimens at all times during preparation. Square gummed labels are obtainable from biological supply houses. Excellent ones are now available with pressure-sensitive cement that prevents the labels from peeling off, as often happens with standard gummed labels that require moisture for attachment . Some workers place all information on one label; others use two labels, one at each end of the slide (fig. 31), with the identification on one label and the collection data on the other. All data should be written clearly with permanent ink, typeset, or typed and reproduced photographically. The kind of mounting medium used should also be noted on a label if remounting is necessary.

Part 4. - Sample Procedures

The following procedures have been successful for general use by specialists in the Systematic Entomology Laboratory. Dissecting, staining, and mounting Lepidoptera genitalia are highly specialized procedures that are not included here. Many other procedures are well adapted for general use, but the simplicity and dependability of the following make them preferred by many specialists.

4.1 - Preparation and Storage of Genitalia.

The structures at the end of the insect abdomen in both sexes are the postabdomen, terminalia, or genitalia, although the last term is more restrictive and refers morphologically only to certain organs of the ninth abdominal segment. These structures, sometimes extending to modifications of many segments of the abdomen, are of great identification importance. Many insects cannot be identified to species without critical examination of these parts, and even then can only be identified in one sex. In some insects, these parts are seen easily without special preparation; in others, just a little special positioning of the genitalia at the time the insects are pinned is sufficient. But in many insect species, these structures are so withdrawn or folded that, for critical examination, the abdomen or a large part of it must be removed and the genitalia specially prepared as follows:

(1) Carefully remove the abdomen by grasping it with forceps as close as possible to the thorax. Bending it slightly upward, then downward, usually will break it free of the specimen. It is well to perform this operation over a small dissecting dish containing water or 70 percent ethanol into which the part can fall. If the specimen is in a fluid and therefore soft, the abdomen may be severed with fine scissors.

(2) Place the severed abdomen in a small beaker or crucible containing three pellets of sodium hydroxide ($NaOH$) in about 10 ml of water. Then set the container on a hotplate at a temperature a little above that needed to boil the solution. It is well to place a cover loosely over the container to prevent the specimen from being thrown out and lost if the solution 'bumps' when heating, or to use a copper-mesh screen between the hotplate and crucible to eliminate 'bumping.' Allow the solution with the specimen in it to boil for 1 minute. Great care must be taken to avoid contact of hot or cold $NaOH$ or caustic with your skin. If that should happen, wash it off immediately with plenty of water.

(3) Remove the specimen with forceps and return it to the dissecting dish. Examine it to see that muscles and most internal organs have been dissolved. If not completely so, return the specimen to the solution and heat it a little more.

(4a) When the specimen is well macerated, take a pair of No. 1 stainless steel insect pins, glued in wooden handles with a drop of epoxy, and pry the genitalia into an extended position. Clear away unwanted parts or debris, or if much unwanted material is present, transfer the specimen to a clean dish of 70 percent ethanol. Water or dilute alcohol is better than glycerin in which to examine small, colorless specimens, partly because fine structures are more clearly visible. The water may be tapwater, but it should be boiled before use to remove dissolved gases that may collect on and in the specimen and be very difficult to remove. The specimen then may be examined and identified or, if necessary, it may be placed in an aqueous solution of a few grains of dry Mercurochrome for staining (see p. 38). The specimen may be held at various angles with a bent piece of minuten and even sketched. If it is to be preserved for permanent reference, the decision must be made whether to store it in a microvial or to mount it on a microscope slide.

(4b) Another useful method when several specimens are to be identified simultaneously includes using a 'spot,' 'well,' or 'depression' plate, which is a white or black ceramic dish generally with 12 wells on the surface. The same

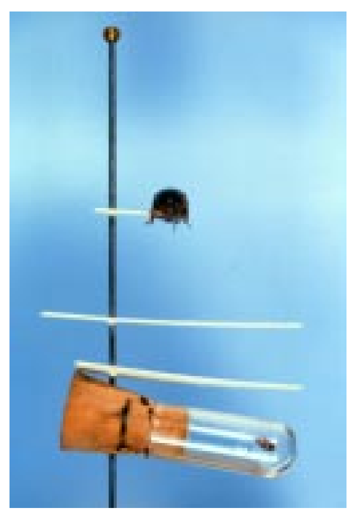


Fig. 32. A point mounted specimen with genitalia vial.

number of wells are utilized as the number of specimens to be identified. (When using more than one specimen, be absolutely certain that the abdomens are properly associated with the correct specimens. To insure this, place the abdomens in the wells in the same order or configuration as the specimens are arranged in their holding container, and mark one side of the plate to indicate its orientation.) To each well add water and one pellet of NaOH with forceps. After the NaOH has dissolved, place one abdomen or part of it in each depression, and warm the plate gently under an incandescent bulb for about 1 hour. After some of the water has evaporated, replace it with fresh distilled water. Also at this time, examine the abdomens and press out any large air bubbles trapped within that might prevent penetration of the caustic. Then reposition the plate under the bulb. A thin stream of macerated tissues soon will be seen to issue from the abdomens into the fresh solution. After an hour or so, depending on the degree of maceration desired, transfer the abdomens for a few minutes to the wells of a second plate that you have filled with 70-80 percent alcohol to which has been added a small amount of acetic acid to neutralize the caustic. While in these wells, the abdomens may be gently manipulated to remove any remaining tissues. Wash and dry the first plate, place 2 drops of glycerin in each well, top with 70-80 percent alcohol, and transfer the abdomens to this plate, using care to keep them in the proper order. The abdomens may now be examined or left in a clean open place for several days if necessary. The glycerin will not evaporate. If the genitalia are to be permanently preserved, place the parts in a microvial as described on page 40.

Mount the specimen on a microscope slide only if it is relatively flat and all needed characters can be seen in the final position. For example, the ovipositors of fruit flies (Tephritidae) are flat enough that they may be fully extended and the ovipositor and sheath, including spermathecae, can be mounted on a slide with all necessary characters well displayed. The postabdomens of the male tephritids, however, are ill suited to such treatment because they are about as thick as they are wide and must be examined in profile as well as in ventral and posterior view.

(5a) If the specimen is to be mounted on a slide, place it in a small dish of 95 percent ethanol for a short time (1 minute is usually sufficient), then add a drop or more as needed of Euparal on a slide. Remove the specimen from the ethanol and immediately place it in the desired position in the Euparal. Break any large bubbles present before carefully lowering the cover glass. If insufficient Euparal is present to run to the entire circumference of the cover glass, add a little more at the edge of the cover glass until a light pressure on the top of the specimen through the cover glass brings the Euparal to the entire edge. Label the slide and allow it to cure (see p. 40) overnight in a warm oven or for a few days in a clean open place to make it usable. Small bubbles will disappear, and the specimen will become a little more transparent.

With the aid of an ocular grid in the microscope, the genitalia may be examined and even sketched when lying in a small dish of water, which gives more contrast than glycerin to delicate structures that may be difficult to see. The object may be held in place with a minuten bent in the shape of the letter 'L' which is laid over it or pierces it at a convenient place. A bit of petroleum jelly will hold a preparation in place, but the jelly must be dissolved before the specimen is replaced in a microvial or mounted on a slide.

(5b) If the specimen is not suitable for mounting on a slide, it may be kept in a microvial. The best microvials are made of transparent plastic with neoprene stoppers. Those with an inner lip are particularly desirable. The former practice was to use glass microvials with cork stoppers, but the tannin in the cork is injurious both to the specimen and, when wet with the glycerin in which the specimen is kept, to the pin on which the preparation is held. Whatever kind of microvial is used, before placing the specimen in the vial, add just enough glycerin to the bottom to cover the specimen completely. A throwaway injection syringe is excellent for this purpose. It may be kept filled with enough glycerin for many preparations. A small container of squeezable plastic with a fine tubular nozzle is made for modelmakers to dispense plastic cement. It is also an excellent glycerin dispenser. After placing the specimen in the vial, add the stopper. A dull-pointed pin inserted between the stopper and vial allows pressure to escape and prevents droppage of the vial from the stopper, which is to be held by an insect pin, preferably the same pin carrying the specimen from which the genitalia preparation was made (fig. 32). The specimen may be removed from the microvial and reexamined in water or ethanol solution at any time and then replaced.

Reference: Gary & Marston 1976; Robinson 1976 (slide-mounting genitalia of Lepidoptera).

4.2 - Mounting Wings

Wings of many kinds of insects can be mounted on microslides for detailed study or photography. Those covered with scales, such as wings of Lepidoptera and mosquitoes, must first have the scales removed or at least bleached for study of the venation.

Wings are bleached by immersion in an ordinary laundry bleach (sodium hypochlorite solution). Wetting them first with ethanol will activate the bleach. Immersion in the bleach for 1-3 minutes is usually sufficient. As soon as the veins become visible, remove the specimen or part from the bleach and wash it in plain water. It is frequently desirable to remove the scales under water by brushing the

wings carefully with a fine brush or with the tip of a small feather. The descaled wing may then be stained, if desired, in eosin-Y or in an aqueous solution of Mercurochrome for a few to several hours and then washed again. The wing is then ready for mounting as described here, or it may be allowed to dry on a slide, then placed under a cover slip, and the cover slip ringed with fingernail polish or ringing compound to hold it in place.

Wings not needing descaling may be removed from a fresh specimen or one that has had a drop of household ammonia (containing detergent) placed at the base of the wing and allowed to stand in a closed receptacle for about an hour. The wing may be removed with finepointed forceps by piercing the body cuticle surrounding the wing base and then pulling the wing loose. In this way, one may be assured of obtaining the complete wing, even with basal sclerites if desired. The wing is then wet with 70 percent ethanol and placed in plain water for about 10 minutes to soften it. It is often desirable, if the wing is from a dried specimen, to place it in water that is then carefully heated until it barely starts to boil. This will aid in removing air from the larger veins. While the wing is in the water, carefully remove any dirt that may be present with a fine brush, but avoid removing fine hairs and setae. Also remove any unwanted parts of body cuticle and muscles at the base of the wing.

Then place the wing for about half a minute in 95 percent ethanol while adding a few drops of Euparal (or balsam) to a slide. Remove the wing from the ethanol and immediately place it in the Euparal (or balsam) on the slide. Position the wing as desired, turning it over if necessary and making sure that its basal part is well stretched out. Alternatively, especially with very delicate wings, it is usually better to arrange the wet wing on the bare slide first, then pour the mounting medium on top. Carefully apply a cover glass, touching it to one side of the Euparal first at a slight angle from horizontal to avoid entrapping bubbles. Press the cover glass down on the wing carefully to expand it as much as possible and to force bubbles out of the basal veins and elsewhere. Then cure the slide in a warm oven overnight or in the open, clean air for a few days. Always excercise caution when dealing with recently mounted slides. While the medium may appear dry at the edges, the interior of the slide may remain liquid for some time and tilting or placing the slide on its side may result in movement of the cover slip.

4.3 - Mounting Larvae of Diptera, Coleoptera, Lepidoptera, and Other Groups.

The study of the immature stages of many insects is of great importance for identification purposes, but special techniques are usually needed because of their soft cuticle. Immature insects of most groups are seldom suitable for preservation in a dry condition. A method given here for preparing dipterous larvae may also be used for immatures of some other groups. Dipterous larvae, especially those of the higher Diptera, have mouthparts, a cephalopharyngeal skeleton, anterior and posterior spiracular structures, anal plates, cuticular spicules, and other features that are important for their systematic study, but these parts usually must be examined at high magnification and require special treatment. The larvae of Diptera, Coleoptera, Lepidoptera, and many other groups are best killed in boiling water because it leaves them in good condition for critical examination.

For cursory examination of the internal cephalopharyngeal skeleton, place the larva with no more fluid than will adhere to it in a dissecting dish. Pierce the cuticle in a few places near the anterior end of the larva and apply a few drops of pure liquid phenol there. Be careful not to get any phenol on your skin; wash with water if you do. In a short time the tissues will become as clear as glass. The larva may be returned to 75 percent ethanol after examination, when the tissues will again become opaque.

For more detailed and permanent preparation of larvae, place the larva in water in a dissecting dish and cut the cuticle with fine dissecting scissors along one side, starting close to the anterior end, passing below the lateral spiracle, and continuing almost to the posterior end. Then place the larva in an NaOH solution and boil as described on page 38. When the larva is well macerated, remove the body contents, almost separate the posterior spiracular area from the remainder of the skin, and pull the cephalopharyngeal skeleton a short way out of the body. Place the skin in 95 percent ethanol while adding a few drops of Euparal on a slide. Then put the skin in the Euparal, opened outward so that the cephalopharyngeal skeleton with the mouth hooks lies away from the skin and the posterior spiracular area lies with both spiracles upward. Apply the cover glass and carefully press it into place. This should give a clear view under high magnification of the cephalopharyngeal skeleton in lateral view, the anterior spiracles, all structures of dorsum and venter of one side, anal plates, and posterior spiracles. The last, often somewhat domed or on conical protuberances, may be distorted, but the sunray hairs and relationships of one spiracle to the other should be easily observed.

As with the genitalia, the larval skin is sometimes best preserved in glycerin in a microvial.

Other parts of the insect body, such as antennae, legs, and palpi, may be mounted on slides in Euparal in the same manner as described for the genitalia, wings, and larvae.

The references cited here concern specialized procedures for making slide mounts of lepidopterous genitalia (Hardwick 1950) and methods using Canada balsam (Noyes 1982; Richards 1964) for aphids, scale insects, parasitoids, and various other small insects. For methods to use with mites (Acarina) (Furumizo 1975; Lipovsky 1951, 1953). Further procedures are also given in the Appendix.

References: Hardwick 1950; Richards 1964; Wirth & Marston 1968.

Part 5 - Labeling

To have any scientific value, specimens must be accompanied by a label or labels giving, as a very minimum, information about where and when the specimen was collected, who collected it, and, if pertinent, from what host or food plant. During preparation and mounting, specimens should bear temporary labels with this information, and any time a sample is subdivided, the label must be copied so that every specimen continues to be accompanied by the data. Many collectors keep a field notebook to record more detailed information, such as general ecological aspects of the area, abundance and behavior of the specimens, and any other observations noted at the time of collection.

5.1 - Paper

The paper used for making labels should be heavy enough so that the labels remain flat and do not rotate loosely on the pin. The surface of the paper should be smooth enough to write on with a fine pen. Linen ledger paper, 100 percent rag and of 36-pound weight, is best. Smooth calendered, two-ply bristolboard is also good; it is usually obtainable from art supply stores. Also desirable is a heavy, high rag-content paper, used for professional-grade herbarium sheets; it may be obtained from biological supply houses. Labels made from poor quality paper become yellow and brittle with age, tend to curl, disintegrate in liquid preservatives, and are ,generally unsatisfactory.

5.2 - Ink

The ink should be a good grade of India ink that is permanent and will not "run" if the labels are placed in jars or vials of liquid preservative. Be sure the ink is completely dry before placing the label in the liquid. It is also helpful to use a waterproofing spray (artist's fixative) on the labels after they are dry. India ink is not always available when collecting in the field. However, labels written with a firm hand and with a moderately soft lead pencil are satisfactory. Do not use ballpoint pens or hard lead pencils for labels placed in liquids; the writing soon fades and becomes illegible.

5.3 - Lettered and Printed Labels

Hand lettered labels using technical pens with very fine points are still widely used at many institutions and by many curators. However, printed labels are preferred and are the medium of choice with most collections and collectors. They may be printed with full data or with spaces left blank for the date. Typewritten or computer generated labels may alternatively be photographed with the proper reduction in size and prints made on high quality rag or parchment bond paper as mentioned previously. Photo-offset methods can also produce satisfactory labels from typewritten copy, but the proper paper must be specified. Common off-the-shelf copier paper is not recommended because of its quality and weight.

Over the last ten years, computer generated labels printed by laser printers have become increasingly common. In the last couple of years, the wide availability and declining cost of printers capable of printing at 600 to 1200 dots per inch have made it easier to produce labels in very small point sizes (5 or less). Software that helps in generating this kind of label is widely available, and the ability to print small batches of labels as they are needed has increased the popularity of this method. In general, these labels seem to work well with pinned specimens. However, laser printed labels may not hold up well in fluids and they are quickly deteriorate in the presence of solvents or the vapors of solvents such as ethyl acetate.

5.4 - Size of Labels

One must seek a middle ground between the size of the insect on a pin and the amount of data a label will hold. Because most insects are small and the amount of necessary data takes up considerable space, try to make labels of a certain maximum size and use more than one label if more data are included. Never use more than one side of a label. The maximum size is about 8 by 18 mm, or in 4-point type, 5 lines of 5 pica length, or about 13 capital letters; however, commercial labels can be much smaller. Large beetles and butterflies need larger labels, but avoid so-called "barndoor" labels because they do not hold well on a pin. Even with very small insects, do not skimp on the amount of data just to make a small label. An advantage of a label that exceeds the size of the insect is that if the specimen is accidentally dropped, the label may keep the insect from being damaged. If capital and lowercase letters are used, it is not necessary to use spaces between words, as JBSmith, NewYork, LittleFalls. If there is any chance of ambiguity, it is best to use full spellings if there is sufficient room. With only one line of data, the label should be wide enough so that when the pin is inserted, all data are legible.

5.5 - Label Data

The indispensable data must answer the questions of where, when, and who, in that order and as exactly as feasible. Only the size of the label should limit the amount of data. This kind of data should be given as follows:

(1) Locality. The collection locality should be given in such a manner that it can be found on any good map. Latitude and longitude are preferred and should be as precise are possible. With the advent of the Global Positioning System (GPS) it is now possible, for a small amount of money, to buy hand held devices that will read off the latitude and longitude to within a few hundred feet. In addition, if the place is not an officially named locality, it should be given in terms of approximate direction and distance from such a locality. The Smithsonian Institution (U.S. National Museum) recommends that for localities in the United States and Canada, the name of the State or Province be spelled in capital letters, such as ONTARIO, ALBERTA, MARYLAND, NEW YORK, and SO. CAROLINA. This method should also be used for foreign countries, as ENGLAND, PAKISTAN, GERMANY (WEST), and SRI LANKA. Then, if at all feasible, the next subordinate region should be cited in capitals and lowercase letters, such as counties and parishes in the United States and Canada and provinces elsewhere. Here are a few examples, with a virgule (/) indicating the end of a line: ARIZONA/CochiseCo./15kmNEPearce (=15 km northeast of Pearce); NEWFOUNDLAND/ Hermitage Dist./12kmWStAlbans; EGYPT Cairo/SuezRoad 38kmW/ Suez;EGYPT Mud.-AI- /Tahrir22km/SWAbulMatamir; or EGYPT/Mud.-AI- Tahrir/30°05'E,30°15'N. Current two letter abbreviations for States and zip codes should not be used because they are not self explanatory and may not be permanent.

(2) Date. Cite day, month, and year in that order, preferably using the international convention of writing day and year in Arabic numerals and the month in Roman numerals without a line over and under the numerals. It is best to place a period or short dash between each number, for example, 4.VII.1978 (=July 4, 1978), 5.V.1909, 5-V-1909. If a few consecutive days have been spent collecting in one locality but not more than a week, the extreme days may be cited, for example, 5-9.V.1909; or if 3 consecutive nights of light trapping were at one spot, the median day may be cited, as 8.VI11.1984 for trapping done on the nights of the 7th to 9th of August 1984. For reared specimens, the dates of collection of the immature stages and of adult emergence should be cited, as pupa 10.VI.1980, em.24.111.1981, indicating that the pupa was collected on 10 June 1980 and the adult emerged on 24 March 1981.

(3) Collector. Spell the last name of the collector or collectors, using initials for given names if space permits. If the last name is a common one, such as Smith, Jones, or Williams, always include initials, and of a group with more than three collectors, use the leader's name followed by et al.

(4) Other Data. It is especially important to cite hosts of parasites and plant-feeding insects when known. Details of the habitat, such as elevation, ecological type, and conditions of collection, are all important and are usually put on a label in addition to the primary data. Such data are "swept from *Salsola kali*," "Malaise trap", "reared ex human feces," "McPhail trap in orange grove," "at light," "3,200 m," "sandy beach," and "under bark dead *Populus deltoides*." Do not use vernacular names of hosts unless the host is common and widespread, such as orange or horse. If the specific name of a host is not known, at least give the genus. "*Vaccinium* sp." is better than no name or "huckleberry." Even the family name of the host is helpful if no more specific name is available. The presumed nature of the association between insect and plant should be clearly indicated, for example, "Resting on flowers of *Vaccinium* sp." The word "ex" (Latin for "out of") should mean that the insect was observed feeding on or in or was bred from the mentioned plant.

As noted earlier, it is advisable to keep a notebook, in which details of locality, habitat, and other important data are kept. However, the practice of assigning code numbers to specimens or containers of specimens which refer back to field notes should be avoided. The use of such codes, which can only be deciphered by reference to notebooks, often results in collections which contain no other data than codes. Over time, the associated notebooks may become lost or misplaced and as a result, the specimens become virtually useless.

5.6 - Placing the Labels

For double-mounted insects, insert the pin through the center of the right side of the label (fig. 21b), with the long axis of the label oriented in the same direction as the card point. Use care that the pin is not inserted through, and thereby obscuring, the writing on the label. For specimens mounted by direct pinning, the label is centered under the specimen with the long axis of the label coinciding with the long axis of the specimen. The left margin of the label is toward the head of the insect. An exception to this is when specimens have the wings spread, such as Lepidoptera. The label is always aligned transversely, at right angles to the axis of the body, with the upper margin toward the head. Labels may be moved up the pin to the desired height by using a pinning block (fig. 18). The middle step of the block will give about the right height if only one label is used. When more than one label is used,

space the labels on the pin beneath the specimen so that the information on the labels can be read without having to move any of them.

5.7 - Bar Coding

In recent years, collection managers have begun to use bar coding (similar to the bar codes found on food and other products) as a way to manage the masses of label data and retrieve information more efficiently. While specialists differ and what is the preferred placement for bar codes, the bar code is usually the last (bottom) label used. At the National Museum of Natural History in Washington, bar codes are often attached upside down so that a specimen can be picked up and read by the bar code scanner without moving or removing any of the other labels. Both bar code scanners and the bar codes themselves are readily obtainable from various suppliers.

5.8 - Labeling Vials

Material in fluid should be accompanied by a single label large enough to include all data. The label should be written with a moderately soft lead pencil or in India ink and well dried so that it will not dissolve or run when immersed in the liquid. Do not use a ballpoint or felt- tip pen. Hard lead pencil writing becomes illegible in liquid. Do not fold the label. Small specimens may be damaged or lost when the label is removed. Multiple labels or labels small enough to float around in the vial may also damage specimens, and when two labels lie face to face, they cannot be read. Always place labels inside the vial as there is the danger that if left outside a vial, regardless of the method or substance used to affix them, they may become defaced, destroyed, or detached.

5.9 - Labeling Microscope Slides

To label microscope slides, use square labels made expressly for this purpose and obtainable from biological supply houses. Labels with pressure-sensitive cement are now available. They are far superior to the older labels, which often came off. Put as much data on the label as feasible, including the kind of mounting medium used in case remounting is needed. Many workers use a label on each side, reserving one for the species determination (fig. 31). Never put labels on the underside of a slide.

5.10 - Identification Labels

When specimens are sent to an expert for identification, they should be accompanied by permanent collection labels giving all essential data. If associated field notes are available, copies of these should accompany the specimens. When the identification has been made, the scientific name of the specimen and the name of the identifier should be printed on a label associated with the specimen. On pinned specimens, this information is always printed on a separate label placed below the collection label or labels on the same pin. When a series of specimens consists of the same species, the identificaton label is often placed only on the first specimen in the series, with the understanding that all other specimens to the right in that row and in following rows belong to the same species. The series ends with another specimen bearing an identification label. Identifications for specimens preserved in alcohol or on slides may be written on the same label as the collection data or on a separate label, depending on the preference of the collector or person making the identification.

Part 6 - Care of the Collection

If care is taken and a few basic precautions are followed, a collection of insects or mites can be maintained indefinitely. The information given here is general; institutions and individuals will want to adapt materials and procedures to fit their own needs and resources.

6.1 - Housing the Collection

The adoption of standard equipment for housing a collection is advantageous as it assures uniformity of containers when additions are necessary. Standard equipment is obtainable from any of several supply houses.

Material preserved in liquid usually needs no attention other than occasional replacement of preservative and stoppers. Small vials may be stored in racks so that the stoppers are not in contact with the liquid. The use of storage racks for vials expedites rearrangement and examination of the material. Vials should be examined periodically to be sure the specimens do not become dry. If it is not possible to inspect the vials frequently, those containing larvae or large insects should have their stoppers replaced by cotton plugs. Several such vials can be placed upside down in a single large jar filled with preservative. Use of cotton plugs is not recommended for very small or delicate specimens because they may become entangled in the cotton fibers. Jars with screw tops or clamping lids, as are used in home canning, are ideal, but jars specifically designed for museum use can be obtained from biological supply houses. Stoppers of neoprene or other synthetic materials generally are superior to cork stoppers, but good quality cork stoppers are usually preferred to plastic screw tops, which often are easily broken. Many of the newer flanged plastic stoppers are excellent.

Microscope slides are usually stored in wooden or plastic boxes obtainable from biological supply houses. The inner sides of the boxes are slotted to hold the slides vertically and to separate them from one another. Slide

boxes are available in sizes made to hold from 50 to 100 or more slides. If the slides are to be stored vertically, it is important that they be thoroughly cured before storage or the cover glasses may slip. Some workers store the slide boxes on their sides so that the slides rest horizontally. This is especially desirable if the slides are made with Hoyer's medium, which may become soft under very humid conditions. Several slide-filing systems are available from suppliers, but whatever system is used, care should be taken to assure that additional similar equipment will be available in the future for expansion of the collection.

For large slide collections, there are slide boxes or cabinets which contain numberous "drawers" in which slides like in a horizontal configuration.

Small plastic slide boxes, usually made to hold five slides, are convenient for keeping slides in a unit-tray system although it is usually best that pinnned specimens and slides not share the same unit. This is especially desirable when genitalia are mounted on slides, because it is readily apparent to visiting researchers examining the pinned specimens that such slides are available.

Pinned specimens are best kept in one of the types of standard, commercially available insect drawers, available in U.S. National Museum (fig. 33), California Academy of Sciences, Cornell, or Schmitt sizes. Larger collections usually use the unit-tray system, with various sizes of unit trays made to fit into a drawer. The pinning bottoms of both the unit trays and boxes are now generally made of polyethylene foam. The older standard was pressed cork, but that was extremely variable in quality and usually contained enough tannin to corrode pins and eventually to cement the pins firmly into the pinning material. Polyethylene foam is now available in large sheets to be cut to the desired size and cemented into boxes or unit trays.

A serviceable substitute for polyethylene is 6-mm-thick balsa wood boards, obtainable from modelmaker



Fig. 33. A U.S. National Museum drawer with foam-bottomed unit trays.

supply houses. These boards should be individually selected for softness because they are frequently excessively hard. Another good substitute, especially for temporary storage of pinned specimens, is double-thickness corrugated board, which is often used to separate layers or rows of cans in cartons. Single-thickness corrugated board will not hold an insect pin firmly, and the harder board used for making cartons is not usable.

Any box used to store insect specimens must be nearly airtight to keep out museum pests—dermestid beetles, psocids (booklice), and certain other insects—which will quickly devour or at least make a shambles of a collection. These pests find their way even into the best boxes or insect drawers, and constant vigilance is necessary.

6.2 - Protecting Specimens From Pests and Mold

Freezing of storage containers is the safest method of fighting or preventing infestations of insect pests such as dermestid beetles inside containers. Containers should be put in a heavy polyethylene bag and placed in the freezer for a period or about 2-5 days at a temperature of -20^{0}C to -25^{0}C (-4 to -13F) degrees or colder. The length of time necessary is dependent on the container and any insulation surrounding the specimens. Specimens should be dry so that there is no danger of crystallization. Incoming packages should be frozen as received so that any pests hiding in shipping materials are killed.

Fumigation of all insect storage boxes may be necessary from time to time. The best made insect drawers provide space for chemical fumigants. Two of the most widely used fumigants are paradichlorobenzene (PDB) and naphthalene, both of which are obtainable in balls or flakes. Never mix PDB with naphthalene as they react chemically and produce a liquid that may damage the collection. It should be noted, that most major collections are now moving away from the use of solid fumigants because of health concerns and in some jurisdictions, it is now against regulations to use some fumigants.

Solid fumigants should be used with **caution** when placed in a box of pinned specimens, and under no circumstances should loose material be included. If crystals or flakes must be used, a small quantity should be placed in a little cloth bag or in a pillbox with the top perforated with tiny holes. This container should be pinned firmly into one corner of the box of specimens. Mothballs may be pinned in a box by attaching the mothball to the head of an ordinary pin. This is done by heating the pin and forcing its head into the mothball. When moving boxes, be careful that the mothballs and fumigant containers do not come loose and damage the specimens.

To kill pests that are actively damaging a collection, you may need to use a liquid fumigant, which acts more rapidly than solid fumigants. Examples of liquid fumigants are carbon disulfide, carbon tetrachloride, chloroform, ethyl acetate, and ethylene dichloride. Because liquid fumigants volatilize rapidly, may be flammable, and are toxic to humans, use extreme care. Work outdoors if possible and use some kind of fumigation chamber. A large plastic bag will serve this purpose. A cotton ball, saturated with a liquid fumigant, may be placed in the infested box, which in turn is placed in the fumigation chamber or plastic bag. One day in the chamber usually is sufficient to kill the pests.

To keep museum pests out of Riker mounts and other display cases, sprinkle naphthalene flakes on the cotton when the mount is prepared. Papered specimens should be kept in boxes with PDB or naphthalene.

All fumigants are toxic to humans to some extent, and most of them are highly flammable. Even PDB, commonly sold for household use, is now considered toxic to some degree. Before using any fumigant, it is well to find out as much as possible about its properties.

In general a combination of mechanical control, sanitation, and the use of inert compounds will help prevent populations of pests from increasing. It is important to keep dust and dirt to a minimum by vacuuming and sweeping. Keep plants to a minimum since these can house and supply food to a variety of potential pests. Make sure that all windows are screened and elminate cracks and crevices around doors and heating ducts where possible. Keep food under cover.

To provide additional control around cabinets, etc. several compounds such as diatomaceous earth, boric acid, and juvenile hormone analogs can be used to control pests such as cockroaches and other resident insects.



Fig. 34. A box of specimens being readied for shipment. Note that ample room has been left on all sides of the specimen container for cushioning material..

Another serious problem, especially in moist, warm climates, is mold, a kind of fungus that readily attacks and grows on insect specimens. Once a specimen has become moldy, nothing can be done to restore it. If only a few filaments or hyphae of mold are present on a specimen, they may be removed carefully with forceps or with a fine brush. The specimen then should be dried in a warm oven. Only keeping the collection in a dry place will prevent mold. In humid climates it is sometimes necessary to keep insect and other kinds of collections in rooms with artificial dehumidification. Some microscope-slide mounting media are also subject to molding.

Reference: Dawson 1992; Furth 1995; Kosztarab 1966; Strang 1992.

Part 7 - Packing and Shipping Specimens

In mailing insects and mites, there is always a risk of damaging or losing specimens. By following the recommendations given here, the risk can be greatly reduced.

7.1 - Packing Materials.

Cartons may be of strong corrugated board or other stiff material. Screw-top mailing tubes are good for small items. All containers must be large enough to include ample packing material to minimize the effects of jarring—a minimum of 5 cm on all sides (fig. 34). There are a wide variety of packing materials ranging from shaved wood and crumpled newspapers to foam or starch "peanuts". One of the best materials is the clear plastic sheet material with a regular pattern of bubbles (bubble wrap or blister pack). This is very light weight and has excellent shock-deadening properties.

7.2 - Pinned Specimens.

Pinned specimens should always be placed as in a small box with a foam pinning bottom. The box should be well wrapped and placed in a larger carton with at least 5 cm of lightly packed packing material between it and the carton on all sides.

(1) Use a sturdy pinning box with a good pinning bottom at least 6 mm thick cemented securely to the bottom of the box. The box should have a tight lid or one held in place with a strip of masking tape. Do not mail specimens in an open-top museum tray.

(2) Pin the specimens firmly into the pinning bottom, leaving enough space for easy removal. Place bracing pins on each side of heavy or long-bodied speci-

mens to prevent them from rotating (cartwheeling) on their pins and damaging adjacent specimens. Microvials should have an additional pin at the end of the vial to keep it from coming off its stopper. Vials other than microvials should be wrapped in a box separate from pinned specimens.

(3) Unless the box in which the specimens are pinned is shallow enough so that the heads of the pins almost touch the lid, a piece of firm cardboard should be cut to fit into the box and lie on top of the pins. If there are only a few specimens in the box, a few extra pins should be added near the corners to keep the cardboard level. It is helpful to attach a tab made of a piece of adhesive tape folded double, with the ends left free to attach to the top of the inserted cardboard. The insert may be lifted out by the tab. The space between the insert and the lid of the box should be filled with enough packing material, preferably cotton batting, not excelsior or any shredded or loose material, to keep the insert pressed lightly against the tops of the pins when the lid is in place. This prevents the pins from working loose and wreaking havoc in transit.

(4) If only one or two specimens are being shipped, they may be placed in a straight-sided plastic vial with a press-on or screw-on top. The vial should be of sufficient diameter to hold the labels in a normal position. A cork stopper cut to such a length that its larger end is a little greater in diameter than that of the inside of the vial is pressed tightly into the bottom of the vial. This will provide a good pinning bottom into which one or two pinned specimens may be firmly pressed. Attach the cover of the vial, wrap the vial in enough packing material to hold it firmly in a mailing tube, attach the cover of the mailing tube, and it is ready to ship.

Although it is good practice to fumigate boxes before shipping, **do not** leave loose fumigant in the box with the specimens nor any fumigant balls on pins in containers. They are especially prone to work loose and damage specimens.



Fig. 35. Some commonly used containers for shipping microscope slides.

(5) Type specimens require special precautions. In most cases types should be packaged individually in small boxes and each box covered with a thin plastic wrap or something similar. In this way, if a specimen is damaged, the pieces are confined to a small area and there is no question of what pieces came from what specimen.

7.3 - Specimens in Vials.

The following procedures are recommended for shipping vials:

(1) Fill each vial with liquid preservative. Stopper tightly by holding a pin or piece of wire between the vial and the stopper to permit air or excess fluid to escape, then remove the pin or wire. Make certain that cork stoppers do not have defects that will allow leakage. Screw-top vials should be firmly closed and sealed with a turn and a half of plastic adhesive tape or Parafilm around the lower edge of the cap and part of the vial. There is no need to seal with paraffin; it often breaks loose and will not prevent leakage.

(2) Wrap each vial with cotton, tissue, paper toweling, or similar material. Allow no piece of glass to come into contact with another piece of glass. Several vials may be wrapped together or held with tape or rubberbands as a unit, or they may be placed in a small cardboard box with enough packing to insure that they are not shaken around.

7.4 - Loading Cartons.

After pinned specimens, specimens in vials, or both have been prepared properly, they should be placed in a strong carton large enough to hold at least 5 cm of packing material around all sides including the top and bottom (fig. 34). Use enough packing material to prevent the contents of the carton from moving about, but do not pack the material tightly. It should be resilient enough to absorb shocks and prevent damage to the contents being shipped. One or a few vials may be shipped in a mailing tube as previously described. When shipping more than one box or packet of vials, tie or wrap them together as a unit before placing them in the larger carton. Individual boxes or vials otherwise may easily be overlooked and lost when unpacked. Since vials of specimens in fluid are much heavier than boxes of pinned specimens, cartons containing many vials may be packed somewhat tighter in the carton than those containing only pinned specimens since they tend to remain relatively stationary in the carton. It is not necessary to ship pinned and liquid-preserved specimens in separate cartons, but if there are many of the latter, it is advisable to ship them separately.

7.5 - Shipping Microscope Slides

First of all, make certain that any slide shipped is thoroughly dried and cured. Many slides may appear to be cured when in fact the center is still liquid. Coverslips on slides which are not cured may move during shipment and damage or destroy the specimens. Slides may be shipped in holders made expressly for that purpose and available commercially from biological supply houses (fig. 35). Even in these holders it is advisable to wrap a little soft tissue around each end of each slide so that the cover glass does not come into contact with anything. The slides may also be shipped in standard storage boxes with enough soft tissue around each end of each slide and between the slides and the box lid to prevent movement. The box should then be wrapped to hold the lid down firmly. It may then be treated as described here for pinned and liquidpreserved specimens. The wrapped slide container may also be tied together with units of pinned or liquidpreserved specimens or both and placed in a carton with them. If no slide holders are available, a few slides, each wrapped with tissue, may be tied together at each end with tape, rubberbands, or string, wrapped in strong paper, and placed in a mailing tube or carton with packing material.

7.6 - Shipping Live Specimens.

Most insects and mites intended for a collection or submitted to experts for identification should not be shipped alive. To protect American agriculture, Federal law prohibits the importation and movement of live pests, pathogens, vectors, and articles that might harbor these organisms unless the shipments are authorized by the U.S. Department of Agriculture. If it is necessary to ship live material, be sure to comply with all Federal, State, and local regulations. Shipments of live insect material without valid permits may be seized and destroyed by plant quarantine inspectors. In addition to meeting Federal laws, the shipment of some species must be approved by State officials. For most questions regarding most federal regulations, contact the Animal and Plant Health Inspections Service (APHIS) within the U.S. Department of Agriculture.

Pupae or larvae shipped to be reared elsewhere should be placed in tightly closed containers without vent holes. These insects require a minimum of air and will not suffocate. Pupae preferably should be packed loosely in moist (not wet) moss or similar material. Larvae should be packed with enough food material to last until their arrival. Most beetle larvae and some caterpillars, especially cutworms, should be isolated, since they are rather cannibalistic. To prevent excessive accumulation of frass (fecal material) and moisture, do not overload containers. Plant material held without ventilation tends to become moldy, especially when kept in plastic bags. For this reason, pieces of the host plant bearing such insects as scale insects (Coccoidea) should be partially or completely dried before being placed in a container, or they should be packed in a container such as a paper bag, which will permit drying to continue after closure. Live Heteroptera and other small, active insects are killed easily by excessive moisture in the container. Therefore, it is advisable to provide several tiny vent holes or place a fine mesh screen over one end of the container when shipping such insects.

Some containers designed to hold living insects are strong enough to be shipped without additional packing, but generally the containers should be enclosed in a second carton with enough packing material to prevent damage to the inner carton. In all cases, affix a permit for shipping live insects in a conspicuous place on the outside of the shipping container.

In recent months, regulations concerning the shipment of dead specimens has changed markedly. This is largely in response to concerns about trade in rare or endangered species and "wildlife". Previously, most insects were excluded from the category of "wildlife", but recent rules have been expanded to include insects in this definition. Within the U.S. it is still possible to ship dead insect without special permits. However, shipment of dead insects to foreign countries (or importation of specimens from foreign sources) may now require the filing of U.S. Fish and Wildlife Service Form 3-177. It is adviseable to check with Fish and Wildlife Service officials at the nearest port of entry (usually either a major airport or sea port) to find out what they require as there can be variation from port to port.

It is recommended that all packages be marked "FRAGILE" and that a complete return address be included on the outside of each container. Valuable specimens such as types should be sent registered mail. While this is more expensive than regular parcel post, it allows misdirected packages to be tracked much more readily. It is also advisable to place a label such as "Dead Insects for Scientific Study. No Commercial Value" on the outside of the package.

Acknowledgements

Several people have been very helpful in putting together this revised version of the manual. I am particularly grateful to Tami Carlow, Warren Steiner, Ron Hodges, John Brown, Alma Solis, Al Newton, Chris Thompson, and Dave Smith.

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Appendix

Formulas Distilled water should be used in these formulas if available, but rainwater or bottled drinking water is satisfactory. "Parts" is by volume.

AGA (Alcohol-Glycerin-Acetic Acid) Solution

Parts

Commercial ethanol (ethyl alcohol)-------------- 8
Water --5
Glycerin -- 1
Glacial acetic acid----------------------------------- 1

Barber's Fluid

Commercial ethanol (ethyl alcohol) ------------- 53
Water---49
Ethyl acetate (acetic ether)------------------------ 19
Benzene (benzol) ------------------------------------ 7

Hoyer's Medium

Chloral hydrate ---20
Water---5
Gum arabic (granules) ---------------------------------3
Glycerin---2

Dissolve gum arabic in water at room temperature. Add chloral hydrate and allow to stand for a day or two until all solids have dissolved. Add glycerin. Filter through glass wool. Store in glass-stoppered bottle.

Essig's Aphid Fluid

Lactic acid -- 20
Glacial acetic acid----------------------------------- 4
Phenol (saturated H_2O solution)------------------- 2
Distilled water-- 1

KAAD (Kerosene-Acetic Acid-Dioxane) Solution

Commercial ethanol (ethyl alcohol)--------------10
Glacial acetic acid------------------------------------2
Kerosene---1
Dioxane--1

Mix in the order given. For very soft-bodied larvae, use half as much kerosene or less. Dioxane may be omitted.

Sample Mounting Procedures

The following procedures for mounting certain insects and mites for scientific study are preferred by the Systematic Entomology Laboratory (ARS, USDA). A few of the chemicals indicated by an asterisk (*) in these procedures are hazardous. Carefully investigate their properties to insure their safe use.

Mounting Aphids, Scale Insects, and Aleyrodids. Specimens of aphids, scale insects, and aleyrodids cannot be pinned because of their small size and their tendency to shrivel. The following procedures are recommended:

(1) Place specimen in 10 percent potassium hydroxide (KOH)* solution and heat gently until body contents are softened, or leave in KOH solution at room temperature for up to 48 hours.

(2) Remove specimen from KOH and place in 70 percent ethanol for 5 minutes. Note for aleyrodids: If black specimens have not turned brown at this point, bleach them in peroxide-ammonia solution (1 drop ammonia to 6 drops hydrogen peroxide) until brown. Next place in 95 percent ethanol for 5 minutes; then proceed to step 7.

(3) Remove from 70 percent ethanol and place in Essig's Aphid Fluid. Make incision halfway across body between second and third pairs of legs. Then squeeze a few times to remove and flush out body contents. If feasible, one or two well-formed embryos should be left in bodies of aphids. Excess wax may be removed by placing specimens in tetrahydrofuran*. (NOTE: This is a hazardous chemical and should be used under exhaust hood and in a very well-ventilated place to avoid inhalation of fumes.)

(4) Remove from Essig's fluid and place in 95 percent ethanol for 5 minutes.

(5) Remove from the ethanol and place in acid fuchsin stain for about 5 minutes or until properly stained, then place in 70 percent ethanol for 5 or more minutes to remove excess stain.

(6) Remove from 70 percent ethanol and place in 95 percent ethanol for 5 minutes.

(7) Remove from 95 percent ethanol and place in clove oil; leave for 5 minutes or until specimen appears nonshiny, dull, and flat.

(8) Remove from clove oil and place specimen dorsum upward on a slide in a drop or two of Canada balsam. If mounting three or more specimens on one slide, place them in a row right to left with one specimen ventral side up. Keep all specimens neatly horizontal with heads pointed in same direction.

(9) Put cover slip in place and attach labels, prefer-

ably so that they may be read with heads of specimens toward you.

Mounting Thrips. For a detailed study, mount thrips in Canada balsam as described here. Place each specimen by itself centrally on a slide with wings, legs, and antennae spread for easy observation of structures. Most specimens should be cleared for optimal appearance of surface detail, but a few should be left in their natural color by omitting steps 2 and 3. Rapid identifications may be made from temporary mounts in glycerin or Hoyer's medium, but they usually cause distortion. Excess or used fluids may be removed at each step with a pipes.

(1) Soak specimen for 24 hours in clean 60 percent ethanol to remove collecting fluid.

(2) Macerate in cold 5 percent sodium hydroxide* solution for 30 minutes or up to 4 hours for especially dark specimens.

(3) Wash briefly in 50 percent ethanol and then leave in 60 percent ethanol for 24 hours.

(4) Dehydrate through a series of ethanol solutions: 70 percent for 1 hour, 80 percent for 2 hours, and 100 percent for 10 minutes (change alcohol once). Place in clove oil until clear (30 seconds to 10 minutes). Spread appendages carefully at each stage. Dehydration and clearing may be promoted by puncturing thoracic and abdominal membranes in one or two places with a fine needle.

(5) Place ventral side uppermost on 13-mm cover slip in Canada balsam, then lower slide onto cover slip. This method is easier to control than the usual method of lowering cover slip onto slide with forceps.

(6) Use two labels on slide, one at each side of specimen, with host, locality, altitude, date, and collector's name on right-hand label and determination and sex data on left-hand label.

(7) Cure in oven at 40° C within a few minutes of preparation, or leave for up to 6 weeks for complete curing.

Mounting Mites Other Than Eriophyids. Mites are most easily mounted if collected in AGA solution (see p. 94). Mount those collected in 70-80 percent ethanol on slides as soon as possible. The following procedures do not apply to mites of the family Eriophyidae:

(1) Place drop of Hoyer's medium (see p. 94) in center of clean 1- by 3-inch microscope slide.

(2) Remove mite directly from host, or pour specimens from collecting vial into small casserole, watch glass, or petri dish. Avoid pouring too much fluid from vial into dish; the less fluid, the easier it is to pick out the mites. The mites may be removed from the host or from the fluid by dipping a needle into the Hoyer's medium on the slide and then quickly touching the mite with it.

(3) Place single specimen in medium on slide. Press specimen to surface of slide and spread all legs laterally. Most mites should be mounted dorsoventrally, but males of many species, such as those of the Acaridae and Tetranychidae, should be mounted laterally to allow examination in profile of the specifically characteristic aedeagus. Some mites may require a small body puncture to eliminate the contents. Heavily pigmented mites may be cleared in a solution of lactophenol before mounting. The solution may be heated to hasten clearing.

(4) With forceps, carefully place clean, small (13-mm or smaller) cover slip on mite in Hoyer's medium. Gently press cover slip with forceps to hold mite in position.

(5) Place slide on hotplate set at 65° C and remove it rapidly when single bubble forms in Hoyer's medium. Avoid more bubbling or mite will be displaced.

(6) Turn slide so anterior part of mite is directed toward you.

(7) Place label on slide to right of cover slip; host, locality, collector, date, and serial number data should be shown on this label.

(8) Place slide in oven at 45°-50° C for 24 hours or longer to cure and solidify the Hoyer's medium. The slide must be kept horizontal until the medium is firm and there is no danger of the cover slip moving.

(9) After removing slide from oven, ringing cover slip with additional Hoyer's medium helps to prevent the mount from drying out.